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HOMEOSTASIS ASSOCIATED WITH HETEROZYGOSITY IN THE GENETICS OF TIME OF VAGINAL OPENING IN THE HOUSE MOUSE[1]

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Received July 22, 1954

VARIATION in quantitative characters observed in long inbred strains of any organism may be interpreted as being due to environmental effects. On an adequate scale, the magnitude of variation in such inbred strains should be equal, within the limit of sampling error, irrespective of the means of the different strains. In crosses between true breeding strains, the variation of the F_1 generation should be equal to the variation in the parental strains.

When the variation in quantitative characters is approximately uniform in the parental strains and the F_1 on some scale, it may be possible to perform a genetic analysis using the methods of MATHER (1949). When no such scale is found or when the variation in F_1 is markedly less than the variation in the parental strains, new assumptions must be made. Such a case was found in a genetic investigation of the time of vaginal opening in the house mouse, *Mus musculus*. The non-heritable variation of the F_1 generation was much smaller than that of the parental strains. The non-heritable variation of the F_2 generation and of the first backcross generations to males of either parental strains was also much smaller than that of parental strains, and did not differ in its magnitude from that of the F_1 generation. The non-heritable variation of the second backcross generations to males of either parental strains, however, showed some increase in its magnitude, as compared to the variation observed in the F_1 and the first backcross generations. Even in these second backcross generations, the magnitude of the non-heritable variation was still much smaller than that of the parental strains.

Similar results have previously been reported by LIVESEY (1930) in rats, MATHER (1949, 1950) in Petunia and Primula species, RASMUSSON (1949, 1952) in sugar beets and Drosophila, ROBERTSON and REEVE (1952) in Drosophila, and DOBZHANSKY and WALLACE (1953) in Drosophila. With the exception of LIVESEY, these authors have linked their observations with the effect of different degrees of heterozygosity. It was similarly concluded, after a statistical analysis of the present data, that the magnitude of the non-heritable variation of a generation is associated with its degree of heterozygosity. The smaller variation in the F_1, F_2, and the first and second backcross generations was ascribed to their greater heterozygosity as compared to that of the parental strains. To this effect of heterozygosity DOBZHANSKY and WALLACE (1953) applied the term homeostasis.

[1] From a dissertation submitted to the Graduate School of The Ohio State University in partial fulfillment of the Doctor of Philosophy degree.

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STRAINS OF MICE AND DESIGN OF EXPERIMENT

Long inbred strains of mice, NB, BALB/c, and C57BL/10, which were obtained from The Ohio State University genetics laboratory, were found to differ with respect to the mean time of vaginal opening. The times were about 22 days in BALB/c, about 24 days in NB, and about 36 days in C57BL/10. All mice were kept under as similar conditions as possible. Mated females were checked for pregnancy once each week, and after they were found pregnant they were checked once each day for litters. When a litter was born, the number of animals in the litter and the date of birth were recorded. As soon as a litter reached the age of ten days, the mice were classified for sex and the vagina of each female was examined daily thereafter. The time of vaginal opening was computed from the day of birth to its day of opening.

Crosses were made between NB and C57BL/10 (cross 1), and between BALB/c and C57BL/10 (cross 2). In cross 1 the NB strain was designated as P_1 and the C57-BL/10 as P_2. In cross 2 the BALB/c strain was designated as P_1 and C57BL/10 as P_2. The mating system in cross 1 and cross 2 is similar. Males and females of P_1 were mated to males and females of P_2 to produce F_1's. To produce F_2's, F_1's were mated to F_1's. The first backcross generation, designated as B_1 or B_2, was produced by crossing F_1 females to either P_1 or P_2 males, B_1 being from P_1, and B_2 being from P_2. The second backcross generation, designated as B_{11} or B_{22}, was produced by crossing B_1 or B_2 females to either P_1 or P_2 males, B_{11} being from P_1, and B_{22} being from P_2.

EXPERIMENTAL RESULTS

The experiment was begun in May, 1952 and was concluded in June, 1953, covering a little more than a year's cycle. During this period a total of 1679 female mice were examined. Of these, 132 females were produced by a mating system not mentioned above. (This part of the data will be discussed separately.) The distribution and the mean time of vaginal opening in various generations of the remaining 1547 females are shown in table 1.

TABLE 1

Number of mice, mean times of vaginal opening, and standard error in various generations in cross 1 and cross 2. In cross 1, P_1 = NB, P_2 = C57BL/10; in cross 2, P_1 = BALB/c, P_2 = C57BL/10

Generation	Cross 1			Cross 2		
	Number of mice	$\bar{X}$	$s_{\bar{X}}$	Number of mice	$\bar{X}$	$s_{\bar{X}}$
P_1	86	24.640	0.872	145	22.193	0.659
P_2	111	36.712	0.754	(111)		
F_1	47	29.298	0.652	192	34.797	0.326
F_2	183	29.907	0.399	163	30.736	0.467
B_1	108	29.500	0.625	107	26.738	0.680
B_2	118	31.619	0.423	74	33.811	0.487
B_{11}	49	32.490	1.049	50	21.340	0.807
B_{22}	88	34.375	0.718	26	36.346	0.940
Total	790			757		

Effect of season

The effect of season on the time of vaginal opening is considerable. This may be made clear by figure 1, which shows the distribution of all the individuals in the C57BL/10 strain, arranged according to dates of birth. As seen in the figure, there is a definite tendency for the vagina to open early in summer and late in winter. This may be observed not only in three parental strains but also in every type of generation such as F_1, F_2, B_1, B_2, B_{11}, and B_{22}, except in a few cases where the collection of data covered only a part of a season.

In order to remove this effect from the data, it was assumed that a seasonal variation exists, and that this repeats itself once every year, reaching its minimum point sometime during the warmer season, and maximum point sometime during the cooler season. On this assumption the data were fitted with a sine curve of the form.

$$\hat{Y} = r \sin (X + U) + h,$$

where r is the amplitude, U the origin, and h the constant or mean level of the curve. This may be written in the form of a sine-cosine curve as:

$$\hat{Y} = f \sin X + g \cos X + h, \tag{1}$$

where $r = \sqrt{f^2 + g^2}$, and $\tan U = f/g$. The quantities f and g together determine the amplitude and the origin of the curve. Since this latter form is more amenable to the problem of estimating parameters by the least squares method, it was used for the curve fitting.

The quantity X in the sine-cosine function is an angle measured in degrees such that each day of a year represents a degree. For convenience a 30 day month was used and a month was divided into six parts. Arbitrarily 0 degree was assigned to the first part of June, the period from June 1st to 5th. After this the corresponding number of degrees for any part of any month can easily be found.

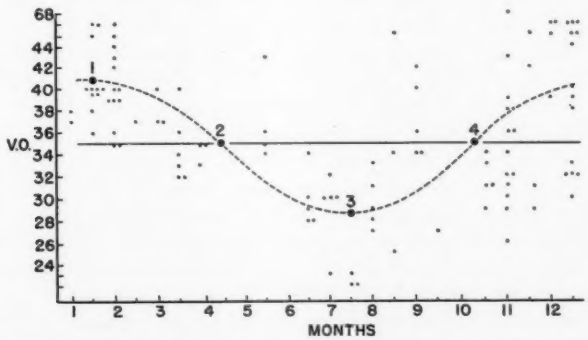

FIGURE 1. The time of vaginal opening arranged according to month of birth, dividing a month into two parts, a and b, in the C57BL/10 strain. The dotted line indicates expected means for various periods of time computed from sine-cosine curve (origin: July 16–20 or $X = 9$). The solid line indicates the constant, h. *1 = point of maximum; *3 = point of minimum; *2 and *4 = points of inflexion.

From equation (1), the following can be written:

$$\hat{Y}_j = f \sin X_j + g \cos X_j + h, \tag{2}$$

where $\hat{Y}_j$ denotes the expected mean for the period X_j. Then let:

$$Q = \Sigma(\hat{Y}_j - Y_{ij})^2 = \Sigma(f \sin X_j + g \cos X_j + h - Y_{ij})^2, \tag{3}$$

such that Q is the quantity to be minimized, Y_{ij} being the observed value for each individual at the time of X_j.

By partial differentiation of the right side of the equation (3) with respect to f, g, and h, and by equating the derivative to zero, three normal equations result. Solving these three normal equations simultaneously, values of f, g, and h can be obtained. Substituting these values of f, g, and h, and the values of X_j in (2), the expected means, $\hat{Y}_j$'s, for various periods of time can be found.

The variance of estimate of $\hat{Y}$ can be computed as:

$$s_Y^2 = \Sigma(Y_{ij} - \hat{Y}_j)^2/df, \tag{4}$$

the standard deviation being $s_Y = \sqrt{s_Y^2}$, and the degree of freedom being N − 3, since three degrees of freedom are sacrificed because of the estimate of f, g, and h.

The variances of quantities f, g, and h may be computed by replacing the right sides of the three normal equations just mentioned, successively by 1, 0, 0; 0, 1, 0; and 0, 0, 1 to give three sets of three equations. Solving these three sets of three equations gives a matrix of multipliers. From this and the variance of Y, the variances of f, g, and h can be obtained.

Tests of whether the seasonal variation is statistically significant were made by using the test of curvilinearity (SNEDECOR 1950). Tests were performed for three parental strains; F_1, F_2, B_1, B_2, B_{11}, and B_{22} in cross 1; and F_1, F_2, B_1, and B_2 in cross 2. From a total of 13 tests, 8 indicated a seasonal effect with significance at the 1% level, and 3 at the 5% level, with only 2 indicating non-significance. It may be concluded that the effect of season on the time of vaginal opening is unmistakable.

The curve with dotted line in figure 1 was constructed by connecting the points of expected means computed by the method described above for various periods of time for C57BL/10 strain. The straight line drawn horizontally in the center of the figure represents the constant, h, around which the curve repeats its cycle. This value of h may now be taken as the mean of the strain.

Fitting the data with the curve becomes especially important when means of two or more different generations, the data of which have been collected at different seasons of a year, are compared. For example, if a mean from data collected during a season of early vaginal opening is compared, without this adjustment, with another mean from data collected during a season of late vaginal opening, the result would lead to biased conclusion. In order to avoid this possible bias, the statistic, h, was used for the comparison of generations.

However, for the generations other than parental strains, fitting the data with the sine-cosine curve (1) was not feasible, since the data of these generations do not

cover a full year. Instead, a sine curve of the following form was used for the purpose,

$$\hat{Y} = f \sin X + h. \tag{5}$$

The principal properties of (5) are the same as (1), except that here the quantity f determines the amplitude of the curve, and the origin of the curve is assigned *a priori*.

The origin of the curves for generations other than parental strains was estimated from the origins of the curves of parental strains. Thus the period between June 26th and 30th, which is the midpoint of origins of NB and C57BL/10, was taken as the origin for all the generations coming from the cross between NB and C57BL/10. For various generations coming from the cross between BALB/c and C57BL/10, the origin of C57BL/10, which is between July 16th and 20th, was chosen as their origin. The reason for not taking the midpoint of the origins of parental strains is that, since the origins of both NB and C57BL/10 are earlier than that of BALB/c, if the midpoint is taken, there may be a risk of fixing the origin in this cross much later than it actually is. Since no adjustment was made of the origin for C57BL/10 in this cross, no adjustment was made for BALB/c either, leaving the origin of the BALB/c strain between July 26th and 31st.

After this decision about the origin of the curves was made, the data for all the generations in cross 1 and cross 2, including those of parental strains, were fitted with the sine curve of (5). The values of h for these generations are shown in table 2.

TABLE 2

*Values of h, variances free from seasonal effect, and variances due to seasonal effect, for P_1, P_2, and subsequent generations in cross 1 and cross 2**

Generation	Cross 1					Cross 2				
	h	s_h	Variances free from seasonal effect	Variances due to seasonal effect		h	s_h	Variances free from seasonal effect	Variances due to seasonal effect	
				Seasonal variance	Average				Seasonal variance	Average
P_1	24.803	0.856	62.627	16.888	9.8365	21.054	0.651	53.231	9.707	14.0425
P_2	34.939	0.700	46.194	2.785		35.110	0.695	44.704	18.378	
F_1	30.154	0.620	15.683	4.270	2.7175	32.340	0.673	19.085	1.297	0.9080
F_2	29.279	0.440	27.917	1.165		28.741	1.058	34.938	0.519	
B_1	27.731	0.800	39.257	3.198	3.0595	25.895	1.554	50.221	−0.759	0.2830
B_2	30.822	0.422	17.878	2.921		31.346	0.988	16.250	1.325	
B_{11}	29.396	1.413	48.761	4.977	10.0690			32.551†		
B_{22}	31.353	0.739	30.341	15.161				22.960†		

* Adjusted origins of curves were used for the computation.

† Unadjusted values: Therefore contain the variances due to seasonal effect.

Effect of age of parents, suckling litter, and size of litters

It was found that the age of parents does not affect the time of vaginal opening of their young. The effect of size of litter was found to be statistically significant. However, no constant correlation was found between the size of litter and the mean time of vaginal opening of three parental strains. So this effect was disregarded in the analysis. The effect of suckling litter was experimentally avoided. Females suckling litters were separated from males, and were not mated until the litters were weaned.

Test of difference between parental strains

The difference between three parental strains was tested using the values of h, computed from unadjusted origins. The difference between NB and C57BL/10, between BALB/c and C57BL/10, and BALB/c and NB were all found to be significant at the 1% level. It was, therefore, concluded that these three strains are genetically different with respect to the time of vaginal opening. No reason was found to suspect that these three parental strains may not be homozygous in loci affecting the time of vaginal opening.

No significant difference was observed between reciprocal F_1's. A significant difference was observed between a backcross generation to males of a parental strain and that to females of a parental strain. This difference will be discussed later.

TEST OF ADEQUACY OF SCALE WITH MEANS OF VARIOUS GENERATIONS

The adequacy of a scale may be tested by comparing the means of various generations. Such a test reveals, in addition, the presence or absence of dominance or balance of dominance. It will also indicate any non-allelic interaction involved in the inheritance of the character tested. Therefore, taking the values of h in table 2 as the means of various generations, adequacy of the time scale was tested, according to the methods described by MATHER (1949).

The results of such tests showed that none of the deviations were significant at the 1% level, but the deviations of B_2 and B_{11} in cross 1, and of B_2 in cross 2 were significant at the 5% level. From these facts it may be concluded that the scale used is in general adequate for both cross 1 and cross 2, and that the gene effects are additive on the scale as a whole, but that there seem to be certain complications which tend to lower the means of backcross generations to P_2, since in both crosses the first backcross means are lower than expected.

PARTITIONING THE VARIANCE

Association between the non-heritable variation and the degree of heterozygosity in various generations

MATHER (1949) divided components of variation in quantitative characters into non-heritable and heritable. The non-heritable variation results from the action of environment and is designated as E. The heritable variation results from the action of genes controlling these quantitative characters, and is divided into fixable and un-fixable.

FISHER, IMMER, and TEDIN (1932) developed a method of determining the con-

tribution of each gene to the fixable and unfixable components. Using the method of these authors, the following relations were found:

$$V_{F_2} = \tfrac{1}{2}D + \tfrac{1}{4}H + E \tag{6}$$

$$V_{B_1} + V_{B_2} = \tfrac{1}{2}D + \tfrac{1}{2}H + 2E \tag{7}$$

$$V_{B_{11}} + V_{B_{22}} = \tfrac{3}{8}D + \tfrac{3}{8}H + 2E \tag{8}$$

$$W_{B_1/B_{11}} + W_{B_2/B_{22}} = \tfrac{1}{4}D + \tfrac{1}{4}H \tag{9}$$

where D denotes the sum of fixable components of variation contributed by all the genes involved, H, the sum of unfixable components of variation also contributed by all the genes involved, and $W_{B_1/B_{11}}$, the covariance between B_1 and B_{11}. In equations (7) and (8) the non-heritable variation, E, is multiplied by 2, because each of the two generations in these equations contributes E to the total variation.

If there is no interaction of the non-heritable components and if the non-heritable variation is independent of genotypes, the quantity E should be the same in all generations within the limit of sampling error. E can be estimated from the magnitude of variation of parental strains and the F_1 generations.

The variance of various generations may be computed by (4) with results shown in table 2 for both cross 1 and cross 2. These variances are, of course, free from variances due to seasonal effect. The variances of the three parental strains do not differ from each other beyond the limit of sampling error. However, the variances of parental strains evidently differ significantly from those of F_1 generations, contrary to expectation. The variances of parental strains are much greater than those of their F_1 generations in both crosses. These differences are both significant at the 1% level. Not only are the variances of the F_1 generations much smaller than expected, but other subsequent generations also show smaller variances than expected.

To clarify these points, a closer examination of the variances of cross 1 will be made. As shown in equation (6), the variance of F_2 is $\frac{1}{2}D + \frac{1}{4}H + E$. It should, therefore, contain the variance due to the non-heritable factors plus variance due to genetic segregation. If the variance of P_1 or P_2 or the average variance of P_1 and P_2 is taken as an estimate of the non-heritable portion of the F_2 variance, the variance of F_2 is evidently too small, since it is smaller than the variance of parental strains. The variances of B_1 and B_2, when examined by the use of the equation (7), show that they are smaller than expected if the non-heritable portion of the variances is estimated from P_1 or P_2 or both. This is true with the variances of B_{11} and B_{22}. The examination of variances in cross 2 yields a similar conclusion. This difference in magnitude of the non-heritable variance can be explained, if it is assumed that the increase of heterozygosity of a generation tends to decrease the magnitude of its non-heritable variance.

However, there are two explanations possible. The first is that the degree of heterozygosity of the generation in question is associated with the magnitude of its non-heritable variance. The second is that the degree of heterozygosity of the parent population of a generation in question is associated with its magnitude. There is evidence that both of these factors are responsible, but for the sake of simplicity, it

will be assumed for this moment that the degree of heterozygosity of the generation in question alone is responsible.

If the heterozygosity of F_1 is taken as unity, the relative heterozygosity of the other generations may be computed as by WRIGHT (1921) as

$$q_{F_1} = 1, \quad q_{F_2} = \tfrac{1}{2}, \quad q_{B_1} = \tfrac{1}{2}, \quad q_{B_2} = \tfrac{1}{2}, \quad q_{B_{11}} = \tfrac{1}{4}, \quad q_{B_{22}} = \tfrac{1}{4} \qquad (10)$$

where q_{F_2} denotes the heterozygosity of the F_2 generation, etc.

The heterozygosity in F_2 is, on the average, $\frac{1}{2}$ of that of F_1. A problem arises as to whether this much of a decrease in heterozygosity increases the non-heritable variance of F_2, as compared to the non-heritable variance of F_1. Since the variance of F_2 contains D and H, it is not clear whether the portion of the non-heritable variance in F_2 is greater than that of F_1. A study of variance due to the seasonal effect, however, has thrown some light on this question. As shown in table 2, the magnitude of the seasonal variance is not the same in all types of generations. There is a clear indication that variance due to seasonal effect is also associated with the degree of heterozygosity of a population. But the variance due to seasonal effect in F_2 in both cross 1 and cross 2 is not greater than that of F_1. Therefore, it was assumed that the loss of $\frac{1}{2}$ of the F_1 heterozygosity does not raise the non-heritable variance in F_2 in both crosses. Since the amount of heterozygosity in B_1 and B_2 is the same, on the average, as in F_2, the value still being $\frac{1}{2}$, it was also assumed that the non-heritable variance does not increase in B_1 and B_2 either, as compared to that of F_1.

The heterozygosity in B_{11} and B_{22}, however, decreases from $\frac{1}{2}$ in F_2 to $\frac{1}{4}$ in these generations. With this decrease of heterozygosity, the variance of B_{11} and B_{22} increases to a considerable degree in cross 1. This increase is also seen in the variance due to seasonal effect. Therefore, it was assumed that the critical point of heterozygosity lies between $\frac{1}{2}$ and $\frac{1}{4}$ of that of F_1 in cross 1, and that when the amount of heterozygosity of any generation passes this critical point, the generation begins to show an increase of the non-heritable variance, as compared to that of F_1. This, however, does not seem to hold true for all types of crosses. In cross 2 the variances of B_{11} and B_{22} do not seem to increase to any considerable degree, as compared to the variance of F_1. The data for these generations in cross 2 were not fitted with the sine curve, because the period in which the data were collected was too short. Therefore, the variances of B_{11} and B_{22} may be a little inflated. This may be expected, because, when any two inbred strains of mice are compared, the degree of heterozygosity may certainly not be the same.

From the above considerations, the non-heritable variance was divided into two portions; one which is residual variance, E_r, obtainable in the generation of maximum heterozygosity, and therefore the minimum variance observed in a group of generations of a cross, and the other, E_i, which is due to an absence of heterozygosity, the maximum value of which is obtained in the long inbred strains. After this, the assumptions made about the association between the degree of heterozygosity and the magnitude of the variance were tested as below.

An estimate of E_r may be obtained from the variance of F_1. This is 15.683 in cross 1. The estimate of E_i may be obtained from the average variance of P_1 and P_2 after

E_r is subtracted. This value in cross 1 is

$$\tfrac{1}{2}(62.627 + 46.194) - 15.683 = 38.7275.$$

Then the components of variances of various generations, and their observed and expected values in cross 1 becomes as shown in table 3. The expected values were found by the methods described by MATHER (1949).

The coefficient of E_i in $V_{B_{11}} + V_{B_{22}}$ was determined as follows. The excess loss of heterozygosity in B_{11} and B_{22}, as compared to F_2, B_1, and B_2 is $\frac{1}{2} - \frac{1}{4} = \frac{1}{4}$. If the critical point is assumed to lie at $\frac{1}{2}$ on the scale of heterozygosity, and the increase of E_i is linearly proportional to the loss of heterozygosity after this critical point, B_{11} and B_{22} will each contribute $\frac{1}{4}E_i$ to the total variance, the summation of the two being $\frac{1}{2}E_i$. For a reason, which will be discussed later, this $\frac{1}{2}$ was multiplied by 2, and the resulting figure 1 was taken as a coefficient of E_i.

From table 3 it can be seen that the deviations of V_{F_2} from expectation is nearly zero. The deviations of the other variances and covariances, E_r, and E_i are not very large. Therefore, it may be concluded that the assumptions tested were given a rather strong support.

The value of H, compared to that of D, is quite large, but, since the standard error of H is large, this value of H may not be dependable. However, since the variance of B_2 is much smaller than that of B_1, and the variance of B_{22} is also smaller than that of B_{11}, a high value of H in P_2 seems to exist. The probable reason for the F_1 mean being so close to the midpoint, instead of being nearer the P_2 mean, will be discussed later.

TABLE 3

Observed and expected variances for various generations with expected values of D *and* H *in cross 1 and cross 2*

Generation	Components of variance	Cross 1			Cross 2		
		Observed variance	Expected variance	Deviation	Observed variance	Expected vraiance	Deviation
V_{F_2}	$\frac{1}{2}D + \frac{1}{4}H + E_r$	27.9170	27.91766	−0.00066	34.9380	34.94013	−0.00213
$V_{B_1} + V_{B_2}$	$\frac{1}{2}D + \frac{1}{2}H + 2E_r$	57.1350	52.63776	4.49724	66.4710	65.60155	0.86945
$V_{B_{11}} + V_{B_{22}}$	$\frac{3}{8}D + \frac{3}{8}H + 2E_r + E_i$	79.1020	82.91674	−3.81474			
$W_{B_1/B_{11}} + W_{B_2/B_{22}}$	$\frac{1}{4}D + \frac{1}{4}H$	5.9920	10.86630	−4.87430	10.2220	11.96856	−1.74656
	E_r	15.6830	17.05136	−1.36836	19.0850	20.83222	−1.74722
	E_i	38.7275	34.91274	3.81476			
Sum of deviations squared				74.96101			6.85920
Expected value of D				6.39514			8.55742
s_D				11.838			4.728
Expected value of H				30.67493			39.31680
s_H				15.334			6.048

Similar tests were made for cross 2. Since in these tests the estimate of E_i could not be tested, only the estimate of E_r was tested. The observed values of variances and covariances of various generations are shown in table 3, together with their expected values. The deviations from expectation are again small, rendering further support to the assumptions tested. The value of H is also high and the variances of backcross generations to P_2 are much smaller than those of backcross generations to P_1. Thus, as in cross 1, the existence of a high value of H in P_2 is strongly indicated.

Effect of degree of heterozygosity of female parents on the magnitude of the non-heritable variance

It was stated previously that 132 females were produced by a mating system not mentioned so far. They were produced in cross 2 by backcrossing males of F_1, B_1, or B_2 to females of parental strains, in contrast to backcrossing hybrid females to parental males. In order to distinguish these two types of backcross generations, the following designations were used. When F_1 females were backcrossed to parental males, the offspring generation was designated as $B_1(1(F_1))$ or $B_2(2(F_1))$, depending on which parental males were used. When F_1 males were backcrossed to parental females, the offspring generation was designated as $B_1((F_1)1)$ or $B_2((F_1)2)$. Designations for the second backcross generations are based on the same principle. The data of the backcross generations so far presented are, of course, those of $B_1(1(F_1))$, $B_2(2(F_1))$, $B_{11}(1(B_1))$, and $B_{22}(2(B_2))$, which were simply referred as B_1, B_2, B_{11}, and B_{22}.

Since animals to be backcrossed to parental strains were chosen at random, two types of B_1, or B_2 should be identical as to their genetic components within the limit of sampling error. The same will be true with B_{11} and B_{22}. The only difference between two contrasting types of generations in any pair is that female parents of one type are from parental strains while those of the other type are hybrids. Therefore, any difference found between two contrasting types of generations may be attributed

TABLE 4

*Means and variances computed from unadjusted data of the first and the second reciprocal backcross generations of cross 2**

Generation	Number of mice	Mean	Variance
$B_1(1(F_1))$	107	26.738	49.462
$B_1((F_1)1)$	27†	28.741	57.500
$B_2(2(F_1))$	74	33.811	17.575
$B_2((F_1)2)$	55†	37.127	69.185
$B_{11}(1(B_1))$	50	21.340	32.551
$B_{11}((B_1)1)$	28†	30.750	120.926
$B_{22}(2(B_2))$	26	36.346	22.960
$B_{22}((B_2)2)$	22†	39.182	66.524

* Since the data of the second backcross generations were collected during a short period of time, no curve fitting was made. It was decided, therefore, to use the unadjusted data for the first backcross generations also.

† These are 132 females produced by the mating system not mentioned in the first part of the discussion.

either to cytoplasmic factors or environmental factors which different genotypes of female parents provide for their young.

The means and variances of various backcross generations are shown in table 4. The difference of means between two contrasting types in B_2, and B_{11} were found to be significant at the 1% level. The difference in B_1 was found to be significant at the 5% level, with only the difference in B_{22} not being significant. The difference of variances between the two contrasting types is also clear in the same table. The difference in B_2, B_{11}, and B_{22} were all found to be significant at the 1% level, with only the difference in B_1 not being significant.

Since the possibility of cytoplasmic factors was eliminated in the tests of reciprocal F_1's, these differences were interpreted as being due to the fact that different degrees of heterozygosity of female parents provide different environmental conditions, which affect means and variances of the distributions of time of vaginal opening of their offspring. The greater the degree of homozygosity of female parents, the greater the increase of both the means and variances of their offspring.

The previous analysis of variance was made upon an assumption that the degree of heterozygosity of parents has no effect upon the variance of their offspring. This assumption is supportable except in the second backcross generations because of the following reasons.

In B_1 and B_2 the female parents were F_1 hybrids, since no data of B_1 or B_2 generation in which female parents were from parental strains was included in the discussion under the assumption. Therefore, no consideration for the absence of heterozygosity of female parents was necessary. However, the situation with the F_1 generation is different since the female parents of this generation are from inbred strains in which the heterozygosity is absent or nearly so. It was assumed, however, that in a generation of such a high degree of heterozygosity as the F_1 in cross 1 and cross 2, the absence of heterozygosity of its female parents does not affect the magnitude of its variance. This assumption was based on the observation of the small seasonal variance of the F_1 generation.

Thus only the second backcross generations, B_{11} and B_{22} present a problem. The female parents of these generations were B_1 and B_2 hybrids. (The second backcross generations whose female parents were from inbred strains were not included in the discussion under the assumption.) In the analysis of variance in cross 1, it was arbitrarily decided, in the absence of definite evidence, that the decrease of heterozygosity in B_1 and B_2 females affects the variance of their offspring in B_{11} and B_{22}, the heterozygosity of which is, on the average, $\frac{1}{4}$. The magnitude of the effect of decrease of heterozygosity in female parents was assumed to be equal to the magnitude of the effect of decrease of heterozygosity in the B_{11} and B_{22} generations themselves. This is why the coefficient of E_i in $V_{B_{11}} + V_{B_{22}}$ in the test of variance of cross 1 was multiplied by 2.

DISCUSSION

The lesser variance of heterozygotes, as compared to the greater variance of homozygotes, may be interpreted as an expression of the better ability of hetero-

zygotes to maintain their own constancy among varying environmental conditions, or of the superior developmental homeostatic functions of heterozygotes compared with those of homozygotes.

DOBZHANSKY and WALLACE (1953) quoted CANNON: "In an open system such as our bodies, compounded of unstable materials and subjected continually to disturbing conditions, homeostasis is in itself evidence that agencies are acting or ready to act, to maintain this constancy." The data of the present report indicate that heterozygosity is one of these agencies.

The homeostatic function of heterozygosity was pointed out by RASMUSSON (1949, 1952), ROBERTSON and REEVE (1952), and DOBZHANSKY and WALLACE (1953). RASMUSSON (1952) stated that heterozygosity increases the resistance of the fly to environmental changes and brings about the greater uniformity of heterozygotes, as compared to homozygotes. DOBZHANSKY and WALLACE (1953) stated that "heterozygotes are more uniformly successful in a variety of environments than are homozygotes; this suggests that the heterozygotes are better able than homozygotes to cope with those different environments and to maintain their internal milieu in functional order."

However, the present data indicate that not only animals themselves but also the developmental environment the female parents provide, which is in turn controlled by genes, play an important role in homeostasis. It appears that not only the lack of heterozygosity in animals themselves leads to a decreased resistance of the animals toward environmental changes but also the lack of heterozygosity in female parents leads to an unfavorable condition for the homeostatic function of their offspring. This unfavorable condition may be overcome to a varying extent if the heterozygosity in the offspring is high enough.

SUMMARY

1. Long inbred strains of house mouse, *Mus musculus*, NB, C57BL/10, and BALB/c were chosen for the study of the inheritance of the time of vaginal opening. During the course of the study it was found that the magnitude of the non-heritable variation of a generation is associated with its degree of heterozygosity.

2. The effect of season on the time of vaginal opening was found to be considerable. In order to remove this effect, a sine-cosine curve (and a sine curve) was fitted to the data.

3. The difference between three parental strains, NB, BALB/c, and C57BL/10 were found to be statistically significant. Therefore, it was concluded that these three strains are genetically different. No difference was found between reciprocal F_1's.

4. Tests of additivity of gene effects showed the scale used is adequate as a whole but with some complications.

5. The following assumptions were made, tested and affirmed about the behavior of non-heritable variation. (a) The magnitude of the non-heritable variation of a generation is associated with its degree of heterozygosity and the heterozygosity of its female parents. (b) The magnitude of the non-heritable variation increases as the degree of homozygosity increases. (c) There is a certain quantitative relation as well

as critical points between the magnitude of the non-heritable variation and the degree of heterozygosity.

6. The lesser variation of heterozygotes, as compared to the greater variation of homozygotes, was interpreted as an expression of the better ability of heterozygotes to maintain their own constancy among varying environmental conditions. In other words, the developmental homeostatic functions in heterozygotes are superior, as compared to homozygotes.

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THE COMPONENTS OF VARIANCE AND THE CORRELATIONS BETWEEN RELATIVES IN SYMMETRICAL RANDOM MATING POPULATIONS[1]*

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THE general problem of the partition of genotypic variance and the correlation between non-inbred relatives in random mating populations was solved by KEMPTHORNE (1954, 1955). The purpose of the present paper is to apply these results to the case of random mating populations for which the genotypic value of a genotype is symmetrical with respect to the components, one from each locus, of the genotype. In the present paper it is supposed that there are only two alleles at each locus with three phases as a consequence, these being specified as the nulliplex $(--)$, simplex $(+-)$ and duplex $(++)$ conditions. Gene frequency will in the present paper be taken to be $1/2$ for every locus. Additional assumptions are normal diploid behavior at meiosis, and no linkage. Attention will be paid particularly to certain classical gene models, which give the genotypic value in terms of the genotype. The ones to be considered are the following: complementary, duplicate factor, multiplicative and optimum number.

THE COMPONENTS OF THE GENOTYPIC VALUE IN SYMMETRICAL POPULATIONS

In general, a genotypic value is expressed in terms of the population mean, the average effect of each gene, a contribution from each pair of allelic genes, a contribution from each pair of non-allelic genes, a contribution from each possible set of three genes and so on.

Following the notation of KEMPTHORNE the n loci of a random mating population will be denoted by superfixes running from 1 to n. At the ith locus it will be supposed that there are m_i alleles which will be denoted by A_j^i where j runs from 1 to m_i. The gene frequency of the jth allele at the ith locus will be denoted by p_j^i. Because of random mating, the genotypic array of the population is

$$\prod_{i=1}^{n} \{\sum p_j^i A_j^i\}^2$$

The symbol $\prod_{i=1}^{n} A_{j_i}^i A_{k_i}^i$ will denote both the genotype of an individual and the genotypic value of that individual. Further definitions follow:

$$\mu = \prod_{i=1}^{n} [\sum_m p_m^i A_m^i][\sum_n p_n^i A_n^i] = \text{mean of population}$$

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$$a^i_{j_i} = [A^i_{j_i} - \sum_m p^i_m A^i_m][\sum_n p^i_n A^i_n] \prod_{i' \neq i} [\sum_m p^{i'}_m A^{i'}_m][\sum_n p^{i'}_n A^{i'}_n]$$

= additive effect of gene $A^i_{j_i}$ in the population

$$d^i_{j_i k_i} = [A^i_{j_i} - \sum_m p^i_m A^i_m][A^i_{k_i} - \sum_n p^i_n A^i_n] \prod_{i' \neq i} [\sum_m p^{i'}_m A^{i'}_m][\sum_n p^{i'}_n A^{i'}_n]$$

= dominance deviation due to genes $A^i_{j_i}$, $A^i_{k_i}$ in the population

$$(a^i a^{i'})_{j_i j_{i'}} = [A^i_{j_i} - \sum_m p^i_m A^i_m][\sum_n p^i_n A^i_n][A^{i'}_{j_{i'}} - \sum_m p^{i'}_m A^{i'}_m],$$
$$[\sum_n p^{i'}_n A^{i'}_n] \prod_{i'' \neq i \neq i'} [\sum_m p^{i''}_m A^{i''}_m][\sum_n p^{i''}_n A^{i''}_n]$$

= additive × additive deviation arising from gene $A^i_{j_i}$ at locus i and gene $A^{i'}_{j_{i'}}$ at locus i',

$$(a^i d^{i'})_{j_i k_i j_{i'} k_{i'}} = [A^i_{j_i} - \sum_m p^i_m A^i_m][\sum_n p^i_n A^i_n][A^{i'}_{j_{i'}} - \sum_m p^{i'}_m A^{i'}_m]$$
$$[A^{i'}_{k_{i'}} - \sum_n p^{i'}_n A^{i'}_n] \prod_{i'' \neq i \neq i'} [\sum_m p^{i''}_m A^{i''}_m][\sum_n p^{i''}_n A^{i''}_n]$$

= additive × dominance deviation arising from gene $A^i_{j_i}$ at locus i, and genes $A^{i'}_{j_{i'}}$, $A^{i'}_{k_i}$ at locus i'.

$$(d^i d^{i'})_{j_i k_i j_{i'} k_{i'}} = [A^i_{j_i} - \sum_m p^i_m A^i_m][A^i_{k_i} - \sum_n p^i_n A^i_n][A^{i'}_{j_{i'}} - \sum_m p^{i'}_m A^{i'}_m]$$
$$[A^{i'}_{k_{i'}} - \sum_n p^{i'}_n A^{i'}_n] \prod_{i'' \neq i \neq i'} [\sum_m p^{i''}_m A^{i''}_m][\sum_n p^{i''}_n A^{i''}_n]$$

= dominance × dominance deviation arising from genes $A^i_{j_i}$, $A^i_{k_i}$ at locus i and genes $A^{i'}_{j_{i'}}$, $A^{i'}_{k_{i'}}$ at locus i'.

$(a^i a^{i'} a^{i''})_{j_i j_{i'} j_{i''}}$ = additive × additive × additive deviation arising from genes $A^i_{j_i}$ at locus i, $A^{i'}_{j_{i'}}$ at locus i', and $A^{i''}_{j_{i''}}$ at locus i'', etc.

When we take the special case of symmetry with gene frequency equal to 1/2 at each locus, we may omit the superscripts for the alleles. We note that any contribution may be expressed formally in terms of genotypic values by multiplying together in appropriate ways factors like

$$\sum p^i_m A^i_m = \left(\frac{A_0 + A_1}{2}\right)$$

and

$$A^i_0 - \sum p^i_m A^i_m = \left(\frac{A_0 - A_1}{2}\right)$$

or

$$A^i_1 - \sum p^i_m A^i_m = -\left(\frac{A_0 - A_1}{2}\right)$$

Hence any contribution to the genotypic values is obtained by multiplying factors $\left(\frac{A_0 - A_1}{2}\right)$ and $\left(\frac{A_0 + A_1}{2}\right)$ and adding the appropriate sign. Hence in the special case being considered,

$$\mu = \left[\frac{A_0A_0}{4} + \frac{2A_0A_1}{4} + \frac{A_1A_1}{4}\right]^n$$

$$a^i_{0_i} = -a^i_{1_i} = \frac{1}{2}\left(\frac{A_0A_0}{2} - \frac{A_1A_1}{2}\right)\left(\frac{A_0A_0}{4} + \frac{2A_0A_1}{4} + \frac{A_1A_1}{4}\right)^{n-1}$$

$$d^i_{0_i0_i} = -d^i_{0_i1_i} = -d^i_{1_i0_i} = d^i_{1_i1_i}$$
$$= \left(\frac{A_0A_0}{4} - \frac{2A_0A_1}{4} + \frac{A_1A_1}{4}\right)\left(\frac{A_0A_0}{4} + \frac{2A_0A_1}{4} + \frac{A_1A_1}{4}\right)^{n-1}$$

$$(a^ia^{i'})_{0_i0_{i'}} = -(a^ia^{i'})_{0_i1_{i'}} = -(a^ia^{i'})_{1_i0_{i'}} = (a^ia^{i'})_{1_i1_{i'}}$$
$$= \left(\frac{1}{2}\right)^2\left(\frac{A_0A_0}{2} - \frac{A_1A_1}{2}\right)^2\left(\frac{A_0A_0}{4} + \frac{2A_0A_1}{4} + \frac{A_1A_1}{4}\right)^{n-2}$$

$$(a^id^{i'})_{0_i0_i0_{i'}0_{i'}} = \pm \text{ similar quantities}$$
$$= \left(\frac{1}{2}\right)\left(\frac{A_0A_0}{2} - \frac{A_1A_1}{2}\right)\left(\frac{A_0A_0}{4} - \frac{2A_0A_1}{2} + \frac{A_1A_1}{4}\right)$$
$$\left(\frac{A_0A_0}{4} + \frac{2A_0A_1}{4} + \frac{A_1A_1}{4}\right)^{n-2}$$

$$(d^id^{i'})_{0_i0_i0_{i'}0_{i'}} = \pm \text{ similar quantities}$$
$$= \left(\frac{A_0A_0}{4} - \frac{2A_0A_1}{2} + \frac{A_1A_1}{4}\right)^2\left(\frac{A_0A_0}{4} + \frac{2A_0A_1}{4} + \frac{A_1A_1}{4}\right)^{n-2}$$

$$(a^ia^{i'}a^{i''})_{0_i0_{i'}0_{i''}} = \left(\frac{1}{2}\right)^3\left(\frac{A_0A_0}{2} - \frac{A_1A_1}{2}\right)^3\left(\frac{A_0A_0}{4} + \frac{2A_0A_1}{4} + \frac{A_1A_1}{4}\right)^{n-3}$$

In general letting $(a^{t_a}d^{t_d})$ be an effect which in its symbolization has a entering t_a times and d entering t_d times, and dropping the subscripts which determine the sign only, we have

$$(a^{t_a}d^{t_d}) = \pm\left(+\frac{1}{2}\right)^{t_a}\left(-\frac{A_0A_0}{2} + \frac{A_1A_1}{2}\right)^{t_a}\left(\frac{A_0A_0}{4} - \frac{2A_0A_1}{4} + \frac{A_1A_1}{4}\right)^{t_d}$$
$$\left(\frac{A_0A_0}{4} + \frac{2A_0A_1}{4} + \frac{A_1A_1}{4}\right)^{n-t_a-t_d}$$

The sign to be attached is essentially irrelevant because we are concerned only with the sum of squares of quantities like $(a^{t_a}d^{t_d})$ with subscripts. A rule can be specified easily.

THE GENE MODELS TO BE CONSIDERED

These are specified as follows, where y_0 denotes the number of $(--)$ loci, y_1 the number of $(+-)$ loci and y_2 the number of $(++)$ loci in the genotype.

Complementary model

$$\text{Genotypic value} = 0^{y_0} = \begin{cases} 0 \text{ when } y_0 > 0 \\ 1 \text{ when } y_0 = 0 \end{cases}$$

An effect is not produced if there are any $(--)$ loci in the genotype. Conversely an effect is produced if the plus gene is present in the simplex $(+-)$ or duplex $(++)$ condition at all loci. A special case of this model (the case in which the number of interacting loci, say n, is equal to *two*) is shown below.

Values of genotypes for complementary model (n = 2)

Phase at second locus	Phase at first locus		
	++	+−	−−
++	1	1	0
+−	1	1	0
−−	0	0	0

Duplicate Factors Model

$$\text{Genotypic value} = 1 - 0^{y_1+y_2} = \begin{cases} 0 \text{ when } y_1 + y_2 = 0 \\ 1 \text{ when } y_1 + y_2 > 0 \end{cases}$$

According to this equation an effect is produced when any locus is in the simplex or duplex phase.

Multiplicative Model

$$\text{Genotypic value} = e^{y_1} b^{y_2}$$

Each locus in the simplex phase results in a multiplication of the genotypic value by e and each locus in the duplex phase results in a multiplication of the genotypic value by b.

Optimum Number Model

The optimum number model is represented by

$$\text{Genotypic value} = -[y_1 + 2y_2 - (n + d)]^2$$

$$= -[d + y_0 - y_2]^2$$

where y_0, y_1, y_2 and n are used as defined elsewhere so that $(y_1 + 2y_2)$ is the number of plus genes of the genotype. The value of the genotype as given above is greatest when $y_1 + 2y_2 = n + d$. Thus $(n + d)$ is an optimum number of plus genes. Since the possible range in number of plus genes is from 0 to $2n$, d may have any value in the range $-n$ to n. The above model represents a special case of a more general one considered by WRIGHT (1935). He distinguished between a primary and a secondary scale for value of a genotype, the latter being a scale of desirability which would measure value in terms of net advantage to the organism. He postulated additivity

of locus effects relative to value on the primary scale, but that maximum value on the secondary scale was associated with an intermediate value on the primary scale.

THE CONTRIBUTIONS TO GENOTYPIC VALUE UNDER THE PARTICULAR MODELS

Any arbitrary contribution apart from sign has been shown above as

$$(a^{t_a}d^{t_d}) = \pm\left(\frac{1}{2}\right)^{t_a}\left(-\frac{A_0A_0 + A_1A_1}{2}\right)^{t_a}\left(\frac{A_0A_0 - 2A_0A_1 + A_1A_1}{4}\right)^{t_d} \times \left(\frac{A_0A_0 + 2A_0A_1 + A_1A_1}{4}\right)^{n-t_a-t_d}.$$

Each of the factors on the right hand side may be expanded by the multinomial theorem thus:

$$\left(-\frac{A_0A_0 + A_1A_1}{2}\right)^{t_a} = \sum \frac{t_a!}{\lambda_{00}!\lambda_{02}!}\left(\frac{A_0A_0}{2}\right)^{\lambda_{00}}\left(\frac{A_1A_1}{2}\right)^{\lambda_{02}}(-1)^{\lambda_{00}},$$

$$\left(\frac{A_0A_0 - 2A_0A_1 + A_1A_1}{4}\right)^{t_d} = \sum \frac{t_d!}{\lambda_{10}!\lambda_{11}!\lambda_{12}!}\left(\frac{A_0A_0}{4}\right)^{\lambda_{10}}\left(\frac{2A_0A_1}{4}\right)^{\lambda_{11}} \times \left(\frac{A_1A_1}{4}\right)^{\lambda_{12}}(-1)^{\lambda_{11}},$$

and

$$\left(\frac{A_0A_0 + 2A_0A_1 + A_1A_1}{4}\right)^{n-t_a-t_d} = \sum \frac{(n - t_a - t_d)!}{\lambda_{20}!\lambda_{21}!\lambda_{22}!}\left(\frac{A_0A_0}{4}\right)^{\lambda_{20}}\left(\frac{2A_0A_1}{4}\right)^{\lambda_{21}} \times \left(\frac{A_1A_1}{4}\right)^{\lambda_{22}}$$

where summation occurs over all portions of the primary exponent, t_a, t_d or $n - t_a - t_d$, as the case may be.

Hence we have

$$(a^{t_a}d^{t_d}) = \pm(\tfrac{1}{2})^{t_a}E(-1)^{\lambda_{00}+\lambda_{11}}(A_0A_0)^{\lambda_{00}+\lambda_{10}+\lambda_{20}}(A_0A_1)^{\lambda_{11}+\lambda_{21}}(A_1A_1)^{\lambda_{02}+\lambda_{12}+\lambda_{22}}$$

where E denotes mathematical expectation over the product of the multinomials specified above, and the symbol for a genotype is replaced by the genotypic value corresponding to that genotype. This product of the multinomials can be written as

$$f(\lambda_{00}, \lambda_{02} \text{ etc.}, \lambda_{22}) = \prod_{i=0}^{2}\left[\frac{\theta_i!}{\prod_j \lambda_{ij}!}\prod_j \Delta_{ij}^{\lambda_{ij}}\right]$$

in which

$$\theta = t_a, \qquad \theta_1 = t_d, \qquad \theta_2 = n - t_a - t_d$$

$$\Delta_{02} = \tfrac{1}{2}, \qquad \Delta_{12} = \tfrac{1}{4}, \qquad \Delta_{22} = \tfrac{1}{4}$$

$$\Delta_{00} = \tfrac{1}{2}, \qquad \Delta_{11} = \tfrac{1}{2}, \qquad \Delta_{21} = \tfrac{1}{2}$$
$$\Delta_{10} = \tfrac{1}{4}, \qquad \Delta_{20} = \tfrac{1}{4}$$

Also we have that

$$(A_0A_0)^{\lambda_{00}+\lambda_{10}+\lambda_{20}}(A_0A_1)^{\lambda_{11}+\lambda_{21}}(A_1A_1)^{\lambda_{02}+\lambda_{12}+\lambda_{22}}$$

interpreted as the genotypic value instead of the genotype, is the genotypic value of a genotype having

$(\lambda_{00} + \lambda_{10} + \lambda_{20})$ nulliplex loci

$(\lambda_{11} + \lambda_{21})$ simplex loci

$(\lambda_{02} + \lambda_{12} + \lambda_{22})$ duplex loci

and may be written as $Y_{y_0y_1y_2}$ where

$$y_2 = \lambda_{02} + \lambda_{12} + \lambda_{22}$$
$$y_1 = \lambda_{11} + \lambda_{21}$$
$$y_2 = \lambda_{00} + \lambda_{10} + \lambda_{20}$$

In the case of the complementary model, for instance

$$Y_{y_0y_1y_2} = 0^{y_0}.$$

We may now use elementary methods or the convenient multinomial expectation identity

$$E \prod_{i,j} \rho_{ij}\lambda_{ij} = \prod_i \left(\sum \rho_{ij}\Delta_{ij}\right)^{\theta_i}$$

given by HORNER (1952) to obtain the expectations. We have:

i	j	Δ_{ij}	ρ_{ij}		
2	2	$\frac{1}{4}$	1	$\theta_2 = n - l_a - l_d$	$\sum_j \rho_{2j}\Delta_{2j} = \frac{3}{4}$
	1	$\frac{1}{2}$	1		
	0	$\frac{1}{4}$	0		
1	2	$\frac{1}{4}$	1	$\theta_1 = l_d$	$\sum_j \rho_{ij}\Delta_{ij} = \frac{1}{4}$
	1	$\frac{1}{2}$	-1		
	0	$\frac{1}{4}$	0		
0	2	$\frac{1}{2}$	1		
	0	$\frac{1}{2}$	0	$\theta_0 = l_a$	$\sum_j \rho_{0j}\Delta_{0j} = \frac{1}{2}$

The results are shown in table 1.

TABLE 1

Values for $(a^{l_a}d^{l_d})$ *under various models*

Model	
Complementary	$\pm(\frac{1}{4})^n 3^{n-l_a-l_d}(-1)^{l_d}$
Duplicate factor	$\pm(-1)^{l_a}[0^{l_a+l_d} - (\frac{1}{4})^n]$
Multiplicative	$\pm(\frac{1}{4})^n(b-1)^{l_a}(b-2e+1)^{l_d}(b+2e+1)^{n-l_a-l_d}$
Optimum number	$a^0d^0 = -(d^2 + \frac{1}{4}n)$, $a^1d^0 = \pm d$, $a^0d^1 = \pm\frac{1}{2}$ $a^2d^0 = \pm\frac{1}{2}$, $a^1d^1 = a^0d^2 = 0$, $a^{l_a}d^{l_d} = 0$ for $l_a + l_d > 2$

COMPONENTS OF GENOTYPIC VARIANCE

KEMPTHORNE (1954, 1955) defines the components of genetic variance as shown below.

$$E\left\{\sum_i [a^i_{j_i}]^2\right\} = \tfrac{1}{2}\sigma^2_A$$

$$E\left\{\sum_i [d^i_{j_i k_i}]^2\right\} = \sigma^2_D$$

$$E\left\{\sum_{i<i'} [(a^i a^{i'})_{j_i j_{i'}}]^2\right\} = \tfrac{1}{4}\sigma^2_{AA}$$

$$E\left\{\sum_{i<i'} [(a^i d^{i'})_{j_i j_{i'} k_{i'}}]^2\right\} = \tfrac{1}{2}\sigma^2_{AD}$$

$$E\left\{\sum_{i<i'} [(d^i d^{i'})_{j_i k_i j_{i'} k_{i'}}]\right\}^2 = \sigma^2_{DD}$$

$$E\left\{\sum_{i<i'<i''} [(a^i a^{i'} a^{i''})_{j_i j_{i'} j_{i''}})]\right\}^2 = \tfrac{1}{8}\sigma^2_{AAA}$$

Under the symmetry assumption,

$$E(a^i_{j_i})^2 = Ea^2_j = p_0 a^2_0 + p_1 a^2_1 = p_0 a^2_0 + p_1(-a_0)^2 = a^2, \text{ say.}$$

Likewise,

$$\begin{aligned} E(d^i_{j_i k_i})^2 = Ed^2_{jk} &= p_0 p_0 d^2_{00} + p_0 p_1 d^2_{01} + p_1 p_0 d^2_{10} + p_1 p_1 d^2_{11} \\ &= p_0 p_0 d^2_{00} + p_0 p_1(-d_{00})^2 + p_1 p_0(-d_{00})^2 + p_1 p_1 d^2_{00} \\ &= d^2, \text{ say.} \end{aligned}$$

The components, then, in the symmetrical case reduce as shown below.

$$\tfrac{1}{2}\sigma^2_A = na^2 = \frac{n!}{1!\,0!(n-1)!}\,a^2$$

$$\sigma^2_D = nd^2 = \frac{n!}{0!\,1!(n-1)!}\,d^2$$

$$\tfrac{1}{4}\sigma^2_{AA} = \frac{n(n-1)}{2}(aa)^2 = \frac{n!}{2!\,0!(n-1)!}(aa)^2$$

$$\tfrac{1}{2}\sigma^2_{AD} = n(n-1)(ad)^2 = \frac{n!}{1!\,1!(n-2)!}(ad)^2$$

$$\sigma^2_{DD} = \frac{n(n-1)}{2}(dd)^2 = \frac{n!}{0!\,2!(n-2)!}(dd)^2$$

$$\tfrac{1}{8}\sigma^2_{AAA} = \frac{n(n-1)(n-2)}{6}(aaa)^2 = \frac{n!}{3!\,0!(n-3)!}(aaa)^2$$

$$\tfrac{1}{4}\sigma^2_{AAD} = \frac{n(n-1)(n-2)}{2}(aad)^2 = \frac{n!}{2!\,1!(n-3)!}(aad)^2$$

$$\tfrac{1}{2}\sigma^2_{ADD} = \frac{n(n-1)(n-2)}{2}(add)^2 = \frac{n!}{1!\,2!(n-3)!}(add)^2$$

$$\sigma^2_{DDD} = \frac{n(n-1)(n-2)}{3!}(ddd)^2 = \frac{n!}{0!\,3!(n-3)!}(ddd)^2$$

Letting $\sigma^2_{A^{t_a}D^{t_d}}$ be the component which has the subscript A appearing t_a times and the subscript D appearing t_d times we have

$$\sigma^2_{A^{t_a}D^{t_d}} = \frac{n!}{t_a!\,t_d!(n-t_a-t_d)!}\,2^{t_a}(a^{t_a}d^{t_d})^2$$

Hence we have the results shown in table 2.

TABLE 2

Formulae for components of genotypic variance

Model	Formula
Complementary	$\frac{n!}{t_a!\,t_d!(n-t_a-t_d)!}\,(\frac{2}{16})^{t_a}(\frac{1}{16})^{t_d}(\frac{9}{16})^{n-t_a-t_d}$
Duplicate factor	$\frac{n!}{t_a!\,t_d!(n-t_a-t_d)!}\,2^{t_a}\,[0^{t_a+t_d} - (\frac{1}{4})^n]^2$
Multiplicative	$\frac{n!}{t_a!\,t_d!(n-t_a-t_d)!}\left[\frac{(b-1)^2}{8}\right]^{t_a}\left(\frac{b-2e+1}{4}\right)^{2t_d}\left(\frac{b+2e+1}{4}\right)^{2(n-t_a-t_d)}$
Optimum number	$\sigma^2_A = 2nd^2,\ \sigma^2_D = \frac{n}{4},\ \sigma^2_{AA} = \frac{n(n-1)}{2}$

DISCUSSION OF MODELS

Complementary Model

The components of genotypic variance can be written down for any particular case from the formula in table 2. To illustrate the results we present the actual components for the case of two and three loci.

For the case of two loci, we find $\sigma^2_A = 36/256$, $\sigma^2_D = 18/256$, $\sigma^2_{AA} = 4/256$, $\sigma^2_{AD} = 4/256$, $\sigma^2_{DD} = 1/256$ so that σ^2_G is equal to 63/256. This conforms to the general result [see for example HORNER 1952 and HORNER, COMSTOCK and ROBINSON unpublished] that σ^2_G equals $(3/4)^n - (3/4)^{2n}$, where n is the number of loci. In the case of two loci, the proportion of genotypic variance which is additive is $36/63 = 4/7$. The parent-offspring covariance is 19/256 in accordance with the general formula for n loci of $(10/16)^n - (9/16)^n$ (HORNER 1952). A detailed discussion of some aspects of these formulae is given by HORNER (1952) and HORNER, COMSTOCK and ROBINSON (unpublished). The full-sib covariance is $(41/64)^n - (9/16)^n$. For two loci it is about 24/256, so that twice the full-sib covariance over-estimates the additively genetic variance by about $\frac{1}{3}$. The parent-offspring covariance leads to an over-estimate by 6%.

For the case of three loci, we find $\sigma^2_A = 486/4096$, $\sigma^2_D = 243/4096$, $\sigma^2_{AA} = 108/4096$, $\sigma^2_{AD} = 108/4096$, $\sigma^2_{DD} = 27/4096$, $\sigma^2_{AAA} = 8/4096$, $\sigma^2_{AAD} = 12/4096$, $\sigma^2_{ADD} = 6/4096$, $\sigma^2_{DDD} = 1/4096$ with $\sigma^2_G = 999/4096$ in accordance with the general formula quoted above. The proportion of genotypic variance which is additive is 486/999 or essentially 49%. The parent-offspring covariance is 271/4096. Twice the parent-offspring covariance over-estimates the additively genetic variance by 12%. The full-sib

covariance is approximately 348/4096, so that twice the full-sib covariance over-estimates the additively genetic variance by about 44%.

Duplicate Factor Model

Hence for the case of two loci, we find $\sigma_A^2 = 4/256$, $\sigma_D^2 = 2/256$, $\sigma_{AA}^2 = 4/256$, $\sigma_{AD}^2 = 4/256$, $\sigma_{DD}^2 = 1/256$, and $\sigma_G^2 = 15/256$. Twice the parent-offspring covariance is 6/256 which is 50% greater than σ_A^2. Twice the full-sib covariance is essentially 8/256, which is twice times σ_A^2. Thus in this case the additively genetic variance is widely overestimated by twice the parent-offspring covariance or twice the full-sib covariance.

For the case of three loci, we find $\sigma_A^2 = 6/4096$, $\sigma_D^2 = 3/4096$, $\sigma_{AA}^2 = 12/4096$, $\sigma_{AD}^2 = 12/4096$, $\sigma_{DD}^2 = 3/4096$, $\sigma_{AAA}^2 = 8/4096$, $\sigma_{AAD}^2 = 12/4096$, $\sigma_{ADD}^2 = 6/4096$, $\sigma_{DDD}^2 = 1/4096$, with $\sigma_G^2 = 63/4096$. In general, σ_G^2 for the duplicate factor model is $(1/4)^n - (1/16)^n$, the parent-offspring covariance is $(2/16)^n - (1/16)^n$, and the full-sib covariance is $(9/64)^n - (1/16)^n$. Twice the parent-offspring covariance for three loci is 14/4096 which over-estimates the additive genetic variance by 133%. Twice the full-sib covariance is essentially 21/4096 which is three and one half times the additive genetic variance.

Multiplicative Model

In this case, the results can be illustrated only by choosing values for b and e. For purposes of illustration we consider the cases considered by HORNER (1952) and

TABLE 3

Component and covariance values for the multiplicative model

Component	$b = e = 1.06$ 20 loci	$b = e = 1.1$		$b = e = 1.4$	
		2 loci	3 loci	2 loci	3 loci
A	4.79×10^{-2}	2.89×10^{-3}	5.01×10^{-3}	6.76×10^{-2}	1.71×10^{-1}
D	2.40×10^{-2}	1.44×10^{-3}	2.50×10^{-3}	3.38×10^{-2}	8.57×10^{-2}
AA	1.88×10^{-1}	1.56×10^{-6}	5.42×10^{-6}	4.0×10^{-4}	2.03×10^{-3}
AD	1.88×10^{-1}	1.56×10^{-6}	5.42×10^{-6}	4.0×10^{-4}	2.03×10^{-3}
DD	4.69×10^{-5}	3.91×10^{-7}	1.35×10^{-6}	1.0×10^{-4}	5.07×10^{-4}
AAA	4.64×10^{-7}		1.95×10^{-9}		8.00×10^{-6}
AAD	6.96×10^{-7}		2.93×10^{-9}		1.2×10^{-5}
ADD	3.48×10^{-7}		1.46×10^{-9}		6.00×10^{-6}
DDD	5.80×10^{-8}		2.44×10^{-10}		1.00×10^{-6}
σ_G^2	7.23×10^{-2}	4.34×10^{-3}	7.52×10^{-3}	1.02×10^{-1}	2.62×10^{-1}
$\frac{\sigma_A^2}{\sigma_G^2}$	.663	.666	.665	.661	.655
$\frac{\sigma_A^2 + \sigma_D^2}{\sigma_G^2}$	.994	.999	.998	.991	.982
Cov (Parent-offspring)	2.40×10^{-2}	1.44×10^{-3}	2.51×10^{-3}	3.39×10^{-2}	8.62×10^{-2}
Full-sib Cov.	3.00×10^{-2}	1.81×10^{-3}	3.13×10^{-3}	4.24×10^{-2}	1.08×10^{-1}
2 Cov (Parent-Offspring)/σ_A^2	1.0025	1.0003	1.0005	1.003	1.006
2 Cov (Full-sib)/σ_A^2	1.25	1.25	1.25	1.25	1.26

HORNER, COMSTOCK and ROBINSON (unpublished), i.e. (i) $b = e = 1.1$ (ii) $b = e = 1.4$ and an additional case (iii) $b = e = 1.06$. This latter value was selected by supposing that the range in bushels per acre for a certain variety of corn was 96 to 30 and that twenty pairs of multiplicative genes with complete dominance were responsible for this range. The ratio of the largest to the smallest genotypic value would be then $b^{20} = 3.2$ which yields a value of $b = 1.06$.

Component and covariance values, along with certain ratios are set out in table 3.

The genotypic variance in general is $\left(\frac{b^2 + 2e^2 + 1}{4}\right)^n - \left(\frac{b + 2e + 1}{4}\right)^{2n}$, the parent-offspring covariance (complete dominance) is $\left(\frac{10b^2 + 4b + 2}{16}\right)^n - \left(\frac{3b + 1}{4}\right)^{2n}$ and the full-sib covariance (complete dominance) is $\left(\frac{41b^2 + 14b + 9}{64}\right)^n - \left(\frac{3b + 1}{4}\right)^{2n}$

An examination of table 3 reveals that the epistatic portion of the genotypic variance is rather small. Indeed the additive portion of this variance amounts to about 66% and the additive and dominance portions together account for 99% in the cases examined. The over-estimation of the additive genetic variance by twice the parent-offspring covariance is trivial. The over-estimation in the case of twice the full-sib covariance is 25%, but this is almost entirely due to dominance. The smallness of the epistatic portion of the genotypic variance in the case of the multiplicative model raises serious questions concerning the usefulness of logarithmic scaling in removing epistatic effects. It does not appear that the use of a logarithmic scale instead of an arithmetic scale when there are multiplicative effects of the order here considered will affect appreciably the estimate of heritability in a population, when gene frequency is one half.

Optimum number model

The components for the optimum number model are $\sigma_A^2 = 2nd^2$, $\sigma_D^2 = \frac{n}{4}$, and $\sigma_{AA}^2 = \frac{n(n-1)}{2}$, all others being zero. Twice the parent-offspring covariance overestimates the additive genetic variance by the fraction $\frac{n-1}{8d^2}$ which is to be contrasted to $\frac{2n-1}{16d^2}$ for twice the full-sib covariance. These fractions will be large if d is close to zero.

GENERAL DISCUSSION

Knowledge of the relative magnitudes of the components of genotypic variance, when epistasis is present, is useful in ascertaining the importance of epistasis in the hereditary mechanism, in computing the bias in estimation and prediction procedures which ignore the effects of epistasis and in determining optimum breeding and selection procedures when epistasis is present. The consequences of the simple epistatic models of this paper might be considered as consequences which might be approached as a limit by the more complex models of nature. An important model omitted

from this paper is the threshold model. With this model an effect is produced if the number of plus genes of a genotype exceeds some threshold number, otherwise it is not produced. The threshold model, although it can be expressed mathematically, appears to be more mathematically intractable than the models considered herein. The assumption of a gene frequency of one-half at every locus makes the results applicable strictly only to an F_2 population. The generalization to an arbitrary gene frequency at any locus has been done and is a step toward determining the consequences of selection when epistasis is present.

SUMMARY

KEMPTHORNE (1954, 1955) solved the general problem of the partition of genotypic variance and the correlation between non-bred relatives in random mating populations. These results are applied in the present paper to the case of random mating populations for which the genotypic value of a genotype is symmetrical with respect to the components, one from each locus, of the genotype, and for which gene frequency at each locus is one-half. The following classical gene models were given particular attention and the results discussed for each: complementary, duplicate factor, multiplicative, and optimum number.

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MATING TYPE MUTATIONS IN VARIETY 1 OF PARAMECIUM AURELIA, AND THEIR BEARING UPON THE PROBLEM OF MATING TYPE DETERMINATION[1]

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THE discovery of complementary mating types in *Paramecium aurelia* (SONNEBORN 1937), together with the later finding that the complementary mating types are hereditarily diverse but genically identical (SONNEBORN 1942), raised a fundamental problem in the genetics of this organism: how do genically identical cells become hereditarily diverse mating types?

The general rule of genic identity is shown by all but two of the nearly 200 stocks in SONNEBORN's collection; an entirely homozygous clone can give rise to both mating types following autogamy. Stocks of this sort are called two-type stocks. The two exceptions show that the genic constitution can play a role in control of mating type. Here we deal with one of these two exceptions, stock P of variety 1, which is pure for mating type I and never yields the complementary type II. Stocks of this sort are called one-type stocks.

By suitable crosses SONNEBORN (1939) has shown that the difference between the one-type condition and the more frequent two-type condition is due to genes at a single locus. A dominant allele, $+^{mtI}$, determines the two-type condition, and its recessive allele, mt^I, the one-type condition. Stocks homozygous for mt^I are therefore genetically incapable of producing mating type II.

This paper deals with the analysis of two mutant stocks incapable of producing mating type II, and with the analysis of a genetic character responsible for an altered frequency of the two alternative mating types in stocks genetically capable of producing either mating type I or II. As will be made clear, the two mutant stocks pure for mating type I contain genes apparently similar to that in stock P, while the genetic character responsible for the altered frequency of mating type determination is probably not allelic to the mt^I locus. Following the presentation of the data, an hypothesis dealing with mating type determination and development will be presented.

METHODS AND MATERIALS

The methods used throughout this work, with one exception to be described, follow those set forth by SONNEBORN (1950).

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Stocks P and 90 of variety 1 served as the normal stocks in these experiments. Stock P, as has already been mentioned, is pure for mt^I, while stock 90 of this variety is a normal two-type stock, giving rise to clones of mating type I and II following nuclear reorganization.

Both mutant stocks arose from stock 90. One stock, now designated as stock d-11, was obtained by NANNEY (personal communication) by exposing a single line of descent to heat shock during three successive nuclear reorganizations. At each reorganization macronuclear regeneration (SONNEBORN 1940) occurred. Following the third reorganization a single isolated individual, the first to be studied in this line of descent, gave rise to culture d-11 which has remained pure for mating type I for three years.

The second mutant stock, designated as stock d-40, arose as follows: a single animal from a type II clone was irradiated under a General Electric Germicidal Lamp (dosage equalled 1100 ergs/mm.^{2}) in approximately 0.10 mm. depth of standard bacterized culture fluid contained in a pyrex depression slide. Irradiation was carried out in a dark room with a yellow bulb furnishing the only source of extraneous light. Since the experiments were designed to obtain mutants no attempt was made to avoid the possible further actions of various components in the medium by reisolating the irradiated cell into fresh medium. Following irradiation the slide depression was merely filled with 1 cc of standard bacterized culture fluid and then placed in a metal moist chamber and kept in the dark for 24 hours at 27°C. The single irradiated animal was allowed to give rise by fission to a small culture.

A single animal isolated from this culture gave rise to another small culture in which conjugating pairs were observed and isolated. (This suggests that autogamy occurred during the development of this subculture and so yielded both mating types.) One product of the first fission of one exconjugant gave rise to stock d-40 which has remained pure for mating type I for over two years.

RESULTS

Evidence for the dependence of the one-type condition of stocks d-11 and d-40 upon a single recessive gene

If the one-type condition in the two mutant stocks depends upon a single recessive gene in each, crosses of each stock to a two-type stock should yield the following: 1) the F_1 should be capable of producing both mating types I and II; 2) the backcross to the mutants should result in a 1:1 ratio of one-type to two-type exconjugant pairs; 3) the backcross to the two-type parent should give no segregation; 4) the F_2 by autogamy should segregate in a 1:1 ratio of one-type to two-type clones; and 5) the F_2 obtained by selfing the F_1 should result in a 3:1 ratio of two-type to one-type exconjugant pairs.

The data confirm the hypothesis that both the mutant stocks contain a single recessive gene restricting them to mating type I. Crosses of both mutant stocks to stock 90 resulted in an F_1 showing the two-type condition. The backcross of F_1 clones from the cross of stock d-11 × stock 90 to the mutant parent yielded 97 one-type and 85 two-type exconjugant pairs. Backcrossing the same F_1 to stock 90 re-

sulted in no segregation in the 30 pairs observed. Similarly, a backcross of stock d-40 × stock 90 F_1 clones to stock d-40 yielded 60 one-type and 65 two-type exconjugant pairs, and no segregation was obtained in 30 backcross pairs with stock 90 used as one parent.

In addition, F_2 clones obtained by autogamy in the F_1 of the d-40 × 90 cross yielded a total of 132 one-type clones, and 140 two-type clones (183 clones failed to survive autogamy). Selfing in one F_1 clone resulted in 92 two-type and 29 one-type exconjugant pairs. All these data are reasonably close to the expected ratios, and indicate that stocks d-11 and d-40 contain a single recessive gene restricting each of them to the one-type condition.

Evidence for the allelism of the genes controlling the one-type trait in stocks P, d-11, and d-40

In order to determine whether the genetic basis of the one-type condition in the two mutant stocks was dependent upon the presence in each stock of an allele of *mt*^{I}, stocks d-11 and d-40 were each crossed to stock 90 and an F_1 obtained. The F_1 from each was then crossed to stock P. If the mutant stocks contain an allele of the *mt*^{I} gene a 1:1 segregation of one-type to two-type progeny should be found, since this would be similar to a backcross. Such was the case for both stocks; the F_1 of the d-11 × 90 cross when crossed to stock P yielded an F_2 of 35 one-type and 47 two-type exconjugant pairs, and the F_1 of the d-40 × 90 cross yielded an F_2 consisting of 74 one-type and 82 two-type exconjugant pairs after being crossed to stock P. At the same time the crosses to stock P were carried out, control backcrosses were made to prove the F_1 was heterozygous at the *mt* locus (data given above).

Again the segregation data indicate a 1:1 ratio is found, and give evidence that both stocks d-11 and d-40 contain an allele of the *mt*^{I} gene previously known in stock P.

Evidence for the presence of a dominant modifying factor, In^{I}*, in stock d-40*

Before discussing the further results of crosses of stock d-40 to stock 90, the usual method of scoring for mating type ratios must be described. First, however, the meaning of the term caryonide needs to be recalled. A caryonide is a group of cells containing macronuclei descended from the same ancestral macronucleus. Normally this means descent from one product of the first fission after autogamy or conjugation, for products of the fertilization nucleus give rise independently to two new macronuclei that separate into different cells at the first fission. SONNEBORN (1937) and KIMBALL (1937) have shown that the members of a caryonide are nearly always identical in mating type, but that the two caryonides from a single fertilized cell are often different in mating type. Hence the usual method of scoring is to grow and test all caryonides separately. The modification of this procedure used in the present study, in order to examine larger numbers, was to forego isolation of caryonides, growing the two caryonides from each fertilized cell in one culture, a clone. If the clone proved to be exclusively type I or exclusively type II, it was scored as two caryonides of one or of the other. If only one caryonide of the two from a fertilized

cell dies, this would introduce some error, otherwise not. As such death did not occur with appreciable frequency, the error involved was insignificant.

On the other hand, some error was introduced by scoring the remaining clones. These were the clones which selfed, i.e., the members of one clone conjugated with each other. These are referred to as selfer clones and were scored as composed of one caryonide of type I and one caryonide of type II. The error here results from the fact that some caryonides are themselves selfers; hence, the two caryonides of a selfing clone could be I-II, I-Selfer, II-Selfer, or Selfer-Selfer. The frequency of selfer clones is, however, small and the error is correspondingly small, as the following direct tests show.

Intrastock 90 matings as well as crosses of stock 90 to stock d-40 were made, and the exconjugants separated into caryonides. The mating type of each caryonide was determined in the normal manner. The data were then treated in two ways. First, the actual frequency of mating types I and II as based on the caryonidal data was determined, with the rare selfing caryonides scored as consisting of one half a type I caryonide and one half a type II caryonide. Secondly, the data were pooled so that the two caryonides of a clone were considered as a single clone, and all selfing clones rescored as if they consisted of one type I caryonide and one type II caryonide. Three different experiments were carried out for each cross. In the six crosses the difference between the actual caryonidal frequency of the mating types and that calculated by the clone method was found not to exceed 3% in any single cross, and to be less than 2% for the total of six crosses. Thus, within the limits of a few percent, the method appears justified.

An additional objection, however, which might be raised against the clone method is that there is always a possibility that a few clones scored as being of only one mating type might in reality have been selfing clones in which the selfing was so slight that it escaped detection. Two additional observations argue against this possibility. First, the clones were always held for an additional five or six fissions after being first tested, and again were observed for the presence of selfing pairs. A single conjugating pair was deemed sufficient to score the clone as a Selfer, and the data were corrected accordingly. Secondly, when the exconjugants were raised as caryonides in the experiments cited above, the total frequency of selfing caryonides was found to be low; only 24 of 619 caryonides (3.9%) were Selfers. Therefore, undetected selfing caryonides could have played only a minor role in the differences to be described.

Other factors which might have influenced the frequency with which the alternative mating types were determined are macronuclear regeneration and cytogamy. If either of these nuclear events occurred with an appreciable frequency after a cross involving a pure type I parent, there would be a tendency for more type I progeny to be detected. Consequently, tests were carried out to ascertain whether either process occurred regularly. Acetocarmine staining of the products of the third fission after conjugation of stocks d-40 × 90 showed only apparently normal macronuclei and no regenerating fragments.

Also, in one instance a clone from each separate pair of a d-40 × 90 cross was followed and a subsequent autogamy induced. Every clone showed segregation of

TABLE 1

Total data for crosses of stocks 90, P, and d-40 (data from table 2 included). Mating type determined by clonal method, see text

Cross	Number of experiments	Total type I caryonides	Total caryonides	Total frequency type I caryonides	Range frequency type I caryonides	Percent increase in type I caryonides over values from intrastock 90 matings
d-40 × stock 90	16	582	1477	.39	.31–.58	77
Stock P × stock 90	11	133	553	.24	.20–.31	9
Stock 90 × stock 90	13	223	1021	.22	.12–.28	—

one-type and two-type progeny in the F_2 indicating that cytogamy had not occurred in the initial cross. Apparently neither macronuclear regeneration nor cytogamy occurs with a sufficiently high enough frequency to affect significantly the results which were obtained.

The progeny of the stock d-40 × 90 cross contained a higher proportion of type I caryonides than did the progeny of either the intrastock 90 matings, or the stock P × stock 90 crosses. The data for all experiments carried out with these stocks are summarized in table 1. χ^2 tests for heterogeneity indicate that the differences between the results of the d-40 × 90 and the 90 × 90 as well as between the d-40 × 90 and P × 90 crosses are significant at the 0.001 level. No significant difference between the 90 × 90 and the P × 90 cross in frequency of type I offspring was found (χ^2 equals 1.01; p equals 0.30 with 1 d.f.). It can be stated with confidence that the resulting F_1 of the d-40 × 90 cross contains more type I caryonides than either of the two other crosses.

In order to show the constancy of these differences, nine of the above experiments were carried out with simultaneous matings; i.e., d-40 was crossed to stock 90 at the same time that either intrastock 90 matings were made, or stock P × 90 matings were obtained (table 2). The results of these simultaneous crosses show that in each

TABLE 2

The frequency of type I caryonides in the F_1 of various crosses. All crosses in each experiment carried out and tested simultaneously. (Data obtained by clonal method of scoring, see text)

Cross	90 × 90		P × 90		d-40 × 90	
Experiment No.	No. caryonides tested	Frequency type I	No. caryonides tested	Frequency type I	No. caryonides tested	Frequency type I
1	26	.27	—	—	26	.58
2	104	.24	—	—	106	.38
3	114	.22	—	—	110	.34
4	100	.23	96	.21	86	.36
5	—	—	52	.23	176	.46
6	—	—	22	.23	60	.47
7	—	—	39	.21	38	.39
8	—	—	54	.20	42	.45
9	—	—	49	.31	59	.37

case the frequency with which type I caryonides was determined was highest in the F_1 of the crosses involving stock d-40 as one of the parents.

The finding that stock d-40 consistently gave a higher frequency of type I caryonides among the F_1 of the cross to stock 90 could be explained in two ways; it was possible either that the allele of mt^I already demonstrated for stock d-40 might act slightly differently from that of stock P, or that other genetic factors might be involved. Experiments were therefore carried out to discover whether the genetic basis for the increased frequency of type I in d-40 × 90 hybrids could be separated from the mt^I gene for pure type I carried by d-40.

The procedure was as follows. The two classes of F_2 obtained by autogamy of the F_1 of a d-40 × 90 cross are, as set forth above, one-type clones and two-type clones. Both kinds of clones were analyzed further. Fourteen of the one-type clones, homozygous for mt^I, were separately crossed to stock 90. The frequencies of mating type I obtained in these crosses are listed in table 3, together with the values for concurrent controls (d-40 × 90, 90 × 90). The frequency of type I found in the progeny of these crosses varied from .20 to .42. Four of the clones fell within the range previously mentioned for the 90 × 90 cross (i.e., .12–.28). One clone was close to the upper limit of values for the 90 × 90 cross (= .29), and 9 were within the range previously determined for the d-40 × 90 cross (.31–.58).

Since variations are found among the different F_1's within each type of cross, repeated simultaneous comparisons are necessary to demonstrate any genetic difference between the F_2 clones dealt with here, and the fact that at least two different kinds of one-type clones were obtained was further verified by repeatedly crossing

TABLE 3

The frequency of type I caryonides obtained after crossing various one-type F_2 clones from the d-40 × 90 cross to stock 90. Mating type determined by the clonal method, see text

F_2 clone number	Number of caryonides tested	Frequency of type I caryonides
160	244	.33
124	76	.36
56	208	.36
20	168	.35
24	116	.35
58	112	.32
49	114	.41
47	118	.32
21	104	.42
5	110	.29
35	108	.27
13	86	.28
14	98	.26
37	108	.20
Controls		
90 × 90, F_1	114	.22
d-40 × 90, F_1	432	.34

TABLE 4

The results of repeated crosses of F_2 clones number 47 and 37 to stock 90. (Experiment 1 from table 3.)

	Experiment number							
	1		2*		3*		4*	
F_2 clone number	47	37	47	37	47	37	47	37
Total caryonides tested	118	108	111	116	107	114	120	117
Frequency type I caryonides	.32	.20	.38	.24	.38	.27	.27	.17
Percentage excess of frequency of type I in clone 47 over frequency in clone 37	60		58		41		59	

* The last three experiments were made using caryonidal data instead of the clonal method.

clones number 37 and 47 to stock 90 and comparing the results (table 4). In the four repetitions of the crosses, the frequency of type I clones among the progeny was always higher among the offspring of the cross involving clone 47 than among the progeny of the cross involving clone 37. The results obtained in the original test are repeatable, and the difference between one-type clones in the F_2 is real.

Further evidence bearing on the genetics of the increase in type I caryonides is obtained from the F_2 two-type clones. These clones were allowed to undergo two successive autogamies and the frequency of type I among the exautogamous progeny determined (table 5). The frequency with which three clones gave rise to type I caryonides was found to be a characteristic of the clone, and it is apparent that there are also at least two types of F_2 clones containing the $+^{mtI}$ gene. This indicated that the increased frequency of type I found after the d-40 × 90 cross cannot be due to the mt^I gene in the former, since in the case of the two-type F_2 clones this recessive factor was not present. This characteristic must therefore depend upon some other genetic basis.

The simplest hypothesis consistent with the data is to assume that a dominant modifying gene, termed In^I, is present in stock d-40. This factor, when present in a clone genetically capable of giving rise to either mating type, acts to favor the probability of type I clones being determined. On this hypothesis, the four possible

TABLE 5

Autogamies of selected F_2 clones showing frequency of mating type I. Experiments 1 and 2 represent successive autogamies of each clone. All figures based on caryonidal data

	Clone number					
	10A		19B		26A	
	Experiment number					
	1	2	1	2	1	2
Total caryonides tested	118	108	111	74	119	108
Frequency of type I caryonides	.36	.39	.41	.41	.26	.23

homozygous genotypes are (1) $+^{mtI}$, in^I, for stock 90 and clone 26A; (2) $+^{mtI}$, In^I, for clones 10A and 19B; (3) mt^I, in^I, for stock P and clone 37; (4) mt^I, In^I for stock d-40 and clone 47.

DISCUSSION

Three results of this and other investigations should be considered in order that a generalized hypothesis of mating type determination and development may be proposed. These are: (1) the failure to find stocks pure for the even mating types although stocks pure for the odd mating type have been found in nature and in the laboratory; (2) the genetic modification of mating type ratios as illustrated by the effects of In^I; (3) the usual restriction of the time of mating type determination to a time soon after fertilization.

The failure to find stocks pure for the even mating types

It is a striking feature of the mating type work that of the nearly 200 stocks in SONNEBORN's collection, including the two new stocks here described, only 4 are known to be pure for one mating type and that *all* four of these are of the odd mating type. While the data are limited, there is at present no stock known which is pure for the even mating type.

Three of the four stocks, P, d-11, and d-40 are pure for mating type I and have been shown to contain apparently similar recessive alleles restricting them to mating type I. In addition, stock 38, the only stock of variety 7, is pure for mating type XIII, and this stock may also contain a similar allele, although the data for this latter case are not as complete (BUTZEL unpublished).

Such results might be expected if the $+^{mtI}$ allele were assumed to control a terminal reaction allowing the development of the even mating types. Stocks homozygous for mt^I would thereby possess a genetic block in such a terminal reaction and would, of necessity, remain odd mating types.

The genetic modification of mating type ratios

The genetic factor In^I, in the presence of $+^{mtI}$, acts to influence the determination of mating type I in variety 1 stocks, indicating that the determination of mating type must be under the control of more than a single gene. Although this is the first case in which the action of a modifying factor acting upon mating type determination has been partially analysed, earlier data obtained by SONNEBORN (personal communication) might have led to a similar conclusion. SONNEBORN found that the crosses of different two-type stocks of variety 1 to stock P yielded consistently different frequencies of type I progeny. Stock R crossed to stock P gave 56–67% type I caryonides in the F_1; stock 60 yielded a frequency of 30–48% type I caryonides. Stock 27 appeared similar to the stock 90 used in these experiments in that it yielded 22–26% type I caryonides in the F_1 when crossed to stock P. Thus each stock of variety 1 tested showed a characteristic frequency of type I caryonides.

Further earlier evidence found by SONNEBORN was derived from stock 60. Here the frequency of type I caryonides found after intrastock matings was noted to drop suddenly so that an overall decrease of 10% type I caryonides was noted. In light

of the present discussion this change might be interpreted as a mutation from a condition similar to In^I to that of in^I.

In addition to these purely genetic factors influencing mating type determination, it is known that environmental factors also play a role. For example, temperature (SONNEBORN 1942b), and certain chemicals in the culture medium (BUTZEL unpublished) are known to influence the determination of mating type.

Therefore, it appears probable that not only the *mt* allele present, but also the residual genome of the cell acts to determine mating type. Environmental factors also may play a role, perhaps in influencing the action of the residual genome so that one or the other mating type is favored.

Specifically, it is perhaps not illogical to consider the $+^{mtI}$ gene as determining the mating type of the cell. If the $+^{mtI}$ gene is present and controls a terminal reaction as suggested above, then the mating type of a caryonide would depend upon whether or not this allele was functional. The residual genome, and the environment, would act to influence the probability of this gene becoming functional. Those cells with this gene acting would develop even mating types, while those in which the $+^{mtI}$ gene did not become functional would remain mating type I. Similarly those cells which are homozygous for mt^I would lack the terminal reaction controlled by the dominant allele and therefore are restricted to the odd mating type.

A somewhat similar hypothesis of gene action has been proposed by SONNEBORN (1951) for the antigen traits in *P. aurelia*. There is good evidence that in the antigen system there are many genes present, each controlling the production of a specific serotype, but that only one of these genes is active at a given time. It is also known that environmental factors may play a decisive role in the determination of which of the antigen-controlling genes becomes functional and thereby controls the serotype of the cell.

The usual restriction of the time of mating type determination to a time soon after fertilization

The determination whether $+^{mtI}$ becomes functional would usually occur only at some time following fertilization and prior to the first cell division after fertilization when mating type is segregated. Once this condition became fixed at this time, mating type would remain constant until the next fertilization, and all progeny of the caryonide would remain pure for a particular mating type until the next fertilization when mating type would again be determined. Thus the physiological condition of the cell at the time when mating type is determined must be considered such that it is only at this time that the residual genome can exert its influence upon the probability of the $+^{mtI}$ gene becoming active.

There may be a further parallel between the mating type system in the A varieties and the antigen system in that certain changes of antigen type are also known to occur more frequently at the time of nuclear reorganization (SKAAR 1952). These changes are believed to be correlated with the physiological condition of the cell during the period of nuclear reorganization. Although the case is not strictly comparable in this instance, the data are cited to indicate that changes restricted to the time of nuclear reorganization are not unique to the mating type system, and that

there also appears to exist a different physiological state at the time of nuclear reorganizations.

SUMMARY

1. Two mutant stocks of variety 1, pure for the odd mating type (I) have been analysed and shown to contain an allele of the mt^I gene previously known to restrict stock P of variety 1 to the odd mating type.

2. An additional modifying factor, In^1, acting to determine the frequency with which type I caryonides appear in stocks genetically capable of producing either mating type I or II has been described.

3. A generalized hypothesis of mating type development and determination has been proposed. Mating type is shown to be the result of the interaction of more than one gene-controlled reaction. It is proposed that the $+^{mt\,I}$ gene controls the determination of the even mating types and that the residual genome, plus environmental factors, influences the probability of this allele becoming functional at a critical time in the life history of a caryonide. It is suggested that cells in which $+^{mt\,I}$ has become functional at this time develop the even mating types, while cells in which this gene has not become active remain as odd mating types. The system of mating type determination and development in the A varieties has been compared to the antigen system.

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COMPETITION BETWEEN CHROMOSOMAL ABERRATIONS ASSOCIATED WITH CURLY AND THEIR WILD TYPE HOMOLOGUES IN LABORATORY POPULATIONS OF DROSOPHILA MELANOGASTER[1]

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INVERSIONS are commonly found in natural populations of animals, but translocations rarely occur. While inversions may affect the viability of an individual, favorably as well as unfavorably in certain cases, relatively complete pairing in meiosis can occur, normal gametes are formed, and the fertility of the organism is seldom affected, even when crossing over occurs within long inverted segments of inversion heterozygotes (STURTEVANT and BEADLE 1936; CARSON 1946). In translocation heterozygotes, however, the rearrangement of the chromosomes into new combinations leads to difficulties in meiosis, since the normal homologues pair with the chromosomes in the translocation (for Drosophila and references to other species, see e.g. STURTEVANT and DOBZHANSKY 1930; DOBZHANSKY and STURTEVANT 1931; GLASS 1933, 1935; BROWN 1940; PIPKIN 1940). Segregation in meiosis leads to the formation of some gametes which may be deficient for large segments of genetic material, while other blocks of genes may be duplicated. In most plants and animals, therefore, except those in which segregation is directed, translocations result in low fecundity. In addition to this, most translocations in Drosophila also adversely affect the viability of the flies carrying them, especially if they are present in the homozygous condition (MULLER and ALTENBURG 1930; PATTERSON, STONE, BEDICHEK and SUCHE 1934). For these reasons inversions should stand a much better chance of becoming established in natural populations of animals than translocations, and such is found to be the case.

The fact that translocations do occur and in some animal species are evidently maintained in natural populations, although rarely, makes it of interest to examine the adaptive value of translocations in laboratory populations where they compete with their wild type homologues. This report deals with the experimental study of one such translocation, itself marked with the dominant gene Curly, which is associated with inversions in the second chromosome.

An account of a portion of the work to be presented has been published earlier in abstract form (ERK 1952).

MATERIALS AND METHODS

The cages used in these experiments (fig. 1) are essentially a modification of the general design originally developed by DA CUNHA (1949). Each cage utilizes a battery of six quart-size square milk bottles. The central chamber is formed by an

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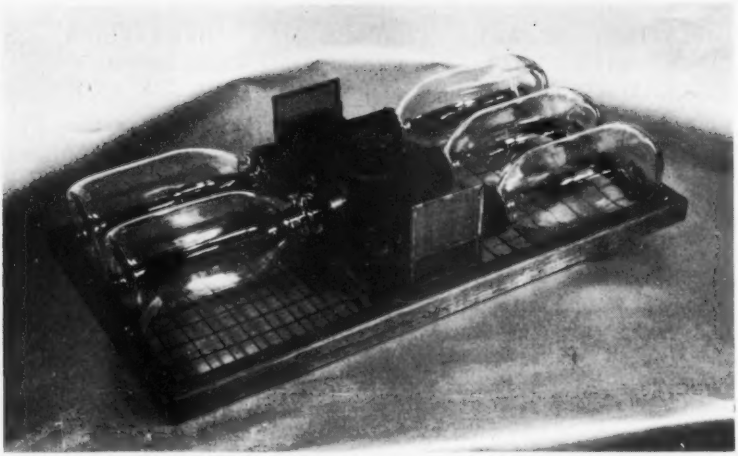

FIGURE 1. View of the population cage, partly disassembled to show the removable end-gates and top port cover, as well as the method of connecting the food bottles.

eleven-inch length of corrugated copper spouting, each end being closed by a removable gate. The gates consist principally of 32-mesh copper gauze, and not only provide for the ventilation of the cage, but also can be easily removed for cleaning the cages between experimental runs. Six lateral ports into the central chamber are provided by short lengths of 1¾ in. O.D. brass tubing soldered to the spouting at proper intervals. A similar piece of tubing is attached to the top of the chamber; the latter is covered by a cap made of a turned piece of brass sheeting, allowing for easy removal or addition of flies to the population. The bottles are securely coupled to the central chamber by the proper length of 1¾ in. automotive radiator hose.

The translocation used was originally obtained by DR. BENTLEY GLASS in an X-irradiation experiment; a second chromosome containing the dominant marker Curly became associated with an unmarked third chromosome in the aberration. The translocation, $T(2;3)^{G9}$ *Cy*, which is lethal when homozygous, has been maintained in a balanced condition for more than ten years, each successive generation being started from *Cy* males and non-translocation females from the same culture. The Curly chromosome used in the control cages was extracted from a stock where it had been used as a balancer for the second chromosome for many years. Curly manifests itself phenotypically by causing the wings to be curled up over the body; it is a dominant associated with inversions in the right and left arms of the second chromosome which strongly suppress crossing over throughout the length of the chromosome, and is lethal when homozygous. The discovery and genetics of Curly wing were reported by WARD (1923).

The Curly translocation was chosen for two reasons. The intimate association of the translocation with an easily classifiable dominant marker permitted large samples and the immediate determination of the frequency of the aberration in the population. Secondly, even though Curly is lethal in the homozygous condition, its pres-

ence as a heterozygote might have a favorable effect on the viability of the fly. The disadvantageous effect of the translocation resulting from lowered viability and partial sterility would be somewhat compensated thereby, and an opportunity would be offered to examine the adaptive value of a translocation when associated with a relatively beneficial gene.

In the control cages identical experiments were conducted, except that Curly in these experiments was not associated with a translocation, but only with the usual inversions in chromosome 2. Hence the parallel experiments serve as a measure of the adaptive value of Curly and its inversions, when competing with its wild type homologue, as well as providing a control for any viability effect that might be due to the inclusion of Curly in the translocation.

Prior to the initiation of the cage experiments, males from the translocation and control stocks, indistinguishable phenotypically in that both showed Curly, were outcrossed to virgin females from the Oregon-R wild type strain. F_1 males were backcrossed to Oregon-R females for a number of generations, so that eventually only the Curly chromosomes of the two stem stocks remained, the rest of the genome being largely replaced by Oregon-R chromosomes. This is not to imply that the genetic backgrounds were isogenic, but only that they were similar with the exception of the chromosomes bearing Curly and the third chromosome which had undergone mutual translocation with the Curly chromosome. The Curly chromosome too goes back to the same origin in both cases, and has kept its identity because of the suppression of crossing over throughout the chromosome.

Because of the recognizability of the heterozygotes, the Curly and wild type flies to make up the initial populations could be collected from the same culture bottles. Curly and wild type males and virgin females were collected from both the translocation and control stocks and maintained in separate containers for a few days before being introduced into the cages. The flies were then etherized, counted, and placed in a single dry vial. When all had recovered, the flies were introduced into the cage through the top port. In all experiments the initial population consisted of 400 individuals, divided equally into Curly (heterozygous) and non-Curly flies, each group being subdivided into equal numbers of both sexes. The initial gametic frequency (p) of the Curly chromosome was therefore 25 percent in both the translocation and control cages.

A single bottle containing standard cornmeal-oatmeal-dextrose-agar medium is attached to the cage at the time the flies are introduced. The initial population invades this bottle, since at this time the other ports are plugged. A fresh food bottle is added weekly, and the population is allowed to expand into the new bottle, since there is free communication in the central chamber between all the attached bottles. With the maximum number of six bottles, the cage will support a population of about 6000 individuals, as determined by actual count. Populations of smaller size could be easily studied by using fewer bottles.

Samples of the adult population were taken at weekly intervals after the population had expanded to its maximum. In the first two or three samples of the experiment, bottles were inverted over the top port and connected by a piece of hose. Those flies that happened to be close would fly or walk into the collecting bottle during the time it was in place, usually a half hour. It was later found that this

method of sampling discriminates against the Curly flies, which are not as adept in regaining a foothold once they have slipped from the sides of the collecting bottle. All later samples were taken at a side port just prior to the introduction of a fresh food bottle there. The collecting bottle was placed in the same position as the food bottles, and the flies were allowed to enter the dry bottle. Two half hour samples were taken in succession for ease of counting, and the results were totaled. The flies from both samples were allowed to recover, and were then introduced into the cage population again through the top port.

The cages were kept in a specially constructed constant-temperature incubator, and the temperature throughout the experiment was maintained at 25° ± 1°C.

RESULTS

The adaptive value of Curly in successive isolated generations

As the Hardy-Weinberg Law is deduced from the assumption of a random union of gametes, it should hold for chromosomal aberrations in populations as well as for single or linked groups of genes. The genetic equilibrium obtains unless disturbed by one or more of the factors capable of altering the frequency of genes (or gene arrangements) in populations, namely, mutation, migration, random genetic drift, and selection. While all of these may play a role in natural populations, it is probable that the changes in gametic frequencies observed in these studies are due primarily to the forces of selection.

In natural populations as well as in the cage studies, generations may overlap when the generation time is sufficiently short. To simplify the determination of the adaptive value of Curly, a complementary experiment was undertaken in which the successive generations were isolated. In the initial generation approximately 50 males and 50 females, all Curly, were placed in a regular culture container. The initial frequency of Curly was therefore 0.5, since all flies showing the character are necessarily heterozygous. The parents were removed to a fresh bottle after eight days, and allowed to remain there for an additional eight days. The first offspring would emerge on the ninth or tenth day (later if the competition was severe), thus excluding the overlapping of generations. The F_1 flies were counted daily and placed in a fresh culture bottle. This procedure was repeated for each generation; except for the smaller sizes of the populations, all conditions were the same as for the cage studies.

From the mating of the Curly heterozygotes of the initial generation, three types of offspring are produced: *Cy/Cy*, *Cy/+*, and *+/+* in the ratio of 1:2:1. Since Curly homozygotes are lethal, only the heterozygotes and the wild type flies survive to produce the next generation. If we assume that selection acts entirely against the homozygotes, and that the heterozygotes and wild type flies are equivalent in their adaptive values, then the frequency of the Curly chromosomes in the $n + 1$ generation is equal to half the frequency of the heterozygotes ($2pq$) divided by the total number of flies following elimination of the *Cy/Cy* flies:

$$p_{n+1} = \frac{p_n q_n}{2p_n q_n + q_n^2} = \frac{p_n}{1 + p_n} \qquad (1)$$

The curve of expected selection against Curly, as determined from formula (1), is the broken line in figure 2. The solid line represents the actual gene frequencies of Curly in 30 successive isolated generations. It will be seen that, subject to some fluctuation, the actual decrease in the frequency of Curly follows the curve of expectation until the 13th generation. From that point on, if we hold the adaptive value of the wild type flies at 1, the relative reproductive fitness of Curly increases, and the frequency of Curly begins to rise again. The rise continues strikingly through the 30th generation.

If we examine the significance of the frequency of Curly heterozygotes at the 15th generation, it is found that not only does the level differ significantly from

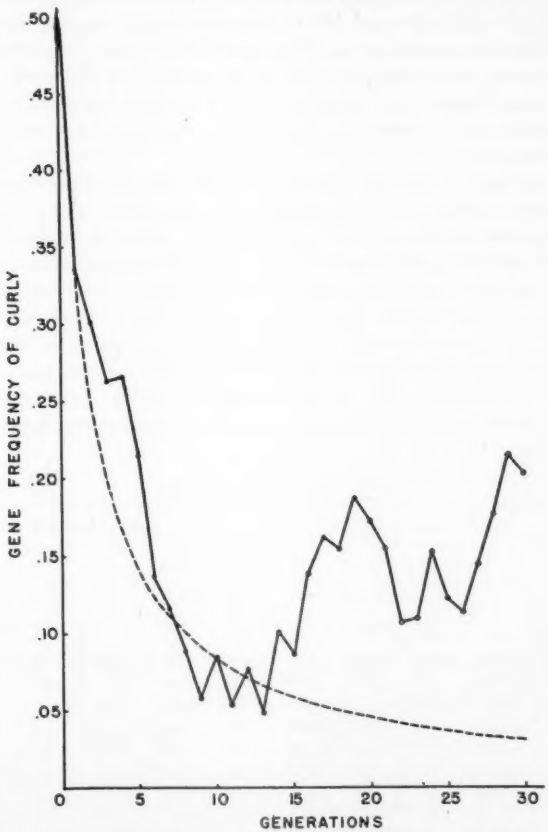

FIGURE 2. The improvement in the adaptive value of Curly heterozygotes in isolated successive generations. The broken line represents the expected frequency, the solid line the actual results obtained.

the low point of the 13th generation ($\chi^2 = 21.09$, d.f. = 1, $P < .01$), but also from the theoretical curve of selection at the 15th generation ($\chi^2 = 19.94$, d.f. = 1, $P < .01$). Since all the values of succeeding generations differ even more than that of the 15th generation from the low point at the 13th generation and from the respective points of comparison on the theoretical curve, the differences must all be highly significant. This emphasizes the real improvement of the adaptive value of Curly heterozygotes, when compared to the wild type; the cause of this reversal in selective value is obscure at present.

The population cage experiments

In the population cage studies, both the experimental and control populations were followed in duplicate experiments conducted simultaneously. The populations were maintained for a period of four months, at which time all the adult flies in the cage were counted and classified except those remaining in the central chamber of the cage; it is estimated that at least 95 percent of the total adult population was counted in each cage at this final census. The latter and the weekly samples are summarized in table 1.

The point in each experiment when collections from the side ports were started is indicated by an asterisk. It will be seen that in cages 1 and 3 the gametic frequency of Curly in the first sample so taken is much greater than in the earlier ones. It must be concluded that the samples taken at the top port earlier did not represent the true gametic frequency of Curly in the population at the time, and all those figures should be considerably higher, thus making the transition from the initial gametic frequency of 25 percent less abrupt. Since quantitative correction of the early values involves assumptions which appear very uncertain, and since the gametic frequencies must have been changing quite rapidly during the period of the early samples, it was decided not to attempt correction but rather to use the later samples as a basis

TABLE 1

Samples from the population cages

Curly Inversion						Curly Translocation					
Cage 1			Cage 3			Cage 2			Cage 4		
Time in days	Number in sample	p	Time in days	Number in sample	p	Time in days	Number in sample	p	Time in days	Number in sample	p
0	400	.2500	0	400	.2500	0	400	.2500	0	400	.2500
32	972	.0206	29	264	.0379	48	961	.0021	44	763	.0066
67	966	.0176	66	213	.0164	67	1386	.0022	65	483	.0031
75	1255	.0080	72	585	.0154	74	706	.0043	71	584	.0069
81	619	.0452*	79	354	.0890*	81	340	.0221*	80	256	.0078*
89	492	.0336	87	309	.0923	89	172	.0087	87	344	.0058
96	739	.0216	95	506	.0603	96	1307	.0023	94	370	.0135
102	323	.0248	102	626	.0765	103	702	.0064	101	404	.0124
110	925	.0146	110	538	.0437	111	1292	.0023	108	703	.0064
117	1614	.0140	117	714	.0525	117	581	.0017	115	897	.0028
123	4709	.0312	125	5709	.0605	124	5888	.0047	124	6769	.0056

* First side port collection.

for determining the probable course of selection against Curly during the earlier part of the experiment.

In determining the expected curve of selection against Curly, if we assume that the Curly homozygote is lethal and that the reproductive fitnesses of $Cy/+$ and $+/+$ flies are equal, the progress of selection against Curly should follow the theoretical curve in figure 2, starting at the 0.25 level.

In calculating the selective disadvantage of the Curly translocation heterozygotes, if we let o represent the orthoploid gametes and a the aneuploid gametes, then six types of gametes will be formed: along one axis of disjunction, o_1 (T-Cy)[1], o_2, a_1 (T-Cy) and a_2, and along the other axis, o_1 (T-Cy), o_2, a_3 (T-Cy) and a_4. The only viable combinations among these gametes are o_1o_2, o_2o_2, a_1a_2, and a_3a_4. In addition to these individuals in the population, viable offspring will result from the union of the orthoploid gametes of the Curly heterozygotes and the gametes of the $+/+$ flies, as well as from the union of gametes from the normal flies alone.

At the start of a given generation, T-$Cy/+$ and $+/+$ flies are present in frequencies X and Y and, it is assumed, interbreed at random. The parent combinations can be summarized in the expression $X^2 + 2XY + Y^2$, representing all possible zygotic combinations. The class X^2 consists of random union of the six types of gametes produced by the Curly heterozygotes. The Y $+/+$ flies will produce $+$ gametes which will combine randomly with the T-$Cy/+$ gametes, as well as with other $+$ gametes. Since the Cy/Cy and inviable aneuploid combinations are eliminated immediately, the frequency of T-$Cy/+$ flies in the $n + 1$ generation can be summarized:

$$X_{n+1} = \frac{2X_n^2(o_1o_2 + a_1a_2 + a_3a_4) + 2X_nY_n(o_1)}{X_n^2(o_2^2 + 2o_1o_2 + 2a_1a_2 + 2a_3a_4) + 2X_nY_n(o_1 + o_2) + Y^2}.$$

Since segregation in a translocation cross-figure is random with respect to a given axis of disjunction (GLASS 1935), we would expect orthoploid and aneuploid gametes to be formed in a ratio of 1:1. If this obtains, then $3X^2/16 + XY/2$ of $X^2/4 + XY + Y^2$ viable flies are Curly translocation heterozygotes. Performing the indicated division and simplifying, the frequency of T-$Cy/+$ flies in the $n + 1$ generation is $(8X - 5X^2)/(16 - 16X + 4X^2)$, or

$$X_{n+1} = \frac{X_n(8 - 5X_n)}{4(X_n - 2)^2}.$$

Since p, the gametic frequency of the Curly translocation gametes, is equal to $X/2$,

$$p_{n+1} = \frac{X_n(8 - 5X_n)}{8(X_n - 2)^2} = \frac{4p_n - 5p_n^2}{8(1 - p_n)^2}. \qquad (2)$$

Formulae (1) and (2) can be used to determine the expected curves of selection against Curly and the Curly translocation. After ten generations of selection against the Curly homozygote, Curly should persist at a gametic frequency of 0.0714; 14 percent of the flies in the population would be phenotypically Curly. On the other

[1] (T-Cy) represents the Curly translocation, although strictly, the aneuploid gametes possess only a part of the translocation.

hand, after ten generations the translocation is expected to be virtually eliminated, with only four gametes in 10,000 carrying Curly. Hence, even though the initial gametic frequency of a translocation might be quite high, it would not be expected to survive in a population unless the disadvantages of low fecundity and viability were somehow compensated.

DISCUSSION

Theoretical considerations on the progress of selection against Curly have assumed that each generation was separable and distinct. Where generations overlap somewhat, as they do in the population cages or in natural populations where there is more than one brood during the fertile period of the parents, the selective value of a particular genotype is more complex, and it would be expected that there would be some difference between the actual and the theoretical courses of selection. In the experiment where successive generations were isolated, the theoretical and actual results differed little through the 13th generation, indicating that, relative to one another, the adaptive values of the Curly inversion heterozygotes and the wild type flies were approximately equal. In the cages, flies capable of extending their fertile period into the next generation would have a reproductive advantage, and this would enhance the overall adaptive value of their genotypes. Since Curly flies tend to get mired more easily than wild type flies in the cages, Curly flies may be at a selective disadvantage in competition when the time period is extended, as in a continuous population.

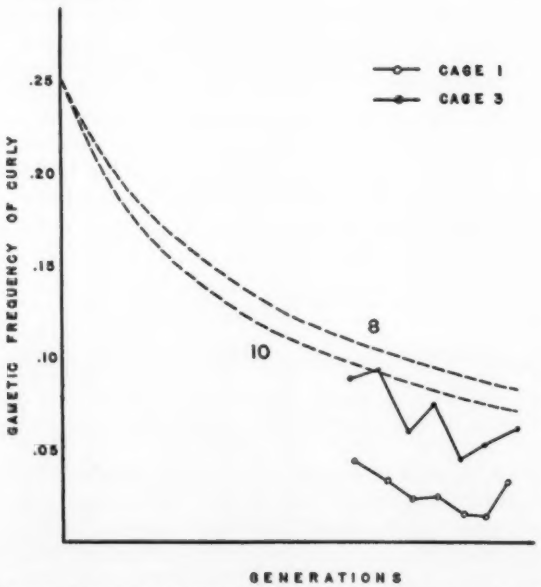

FIGURE 3. The expected curves of selection against Curly homozygotes over 8 and 10 generations, as compared with the actual progress of selection against Curly.

Since successive generations are not separable in the cages, it is necessary to estimate the average length of generation that is approximately equivalent to those of separable generations. At 25°C, the first adults eclose in a fresh food bottle about ten days after the bottle has been added to the cage. Females are most productive early in their reproductive life, and usually reach a peak on the second or third day. In the brood of any given female, presumably zygotes from those eggs which were first laid would have an initial advantage in securing food, so that the "average" fly which replaces a given fly in the population would result from a zygote produced during the first few days by the parent. The average generation time is therefore probably between twelve and fifteen days, and during the course of the experiment (about 120 days), between eight and ten generations would have been completed.

Using these estimates representing the probable limits of the number of generations completed during the course of the experiment, the actual data are plotted against the two theoretical curves of selection; this is done for Curly alone in figure 3, and for the Curly translocation in figure 4. As will be noted from the graphs, the gametic frequency of Curly alone apparently fluctuated somewhat from week to week; although of the same magnitude, the frequencies in the two populations differed consistently throughout the course of the experiment. The Curly chromosome was present at frequencies of 3.12 and 6.05 percent when the experiment ended. The populations containing the Curly translocation differed less widely,

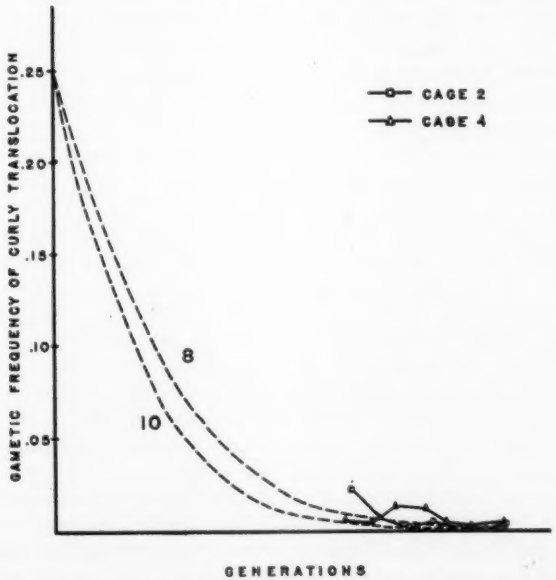

FIGURE 4. The expected curves of selection against the Curly translocation over 8 and 10 generations, as compared with the actual progress of selection against the translocation.

and when the experiment was terminated their gametic frequencies were almost identical, 0.47 and 0.56 percent respectively.

The comparison of the data curves with the theoretical ones emphasizes the similarity between the expected and the actual results. The data from cage 1 are at variance with the general picture, of course, and the difference between the final gametic frequencies of Curly in cages 1 and 3 is statistically significant. Such results in a single cage might be obtained if there had been for some reason unusually rigorous selection against the Curly heterozygotes in the initial generation when the total population was small. The successive samples from cage 1 show good consistency, and it seems obvious that the selective elimination of the Curly flies must have occurred prior to the first sample. The curve for the frequency of Curly in cage 1 clearly belongs to the same family of curves as the others, but inasmuch as it differs significantly from that of cage 3, it seems advisable to use chiefly the results of cages 2, 3 and 4 in drawing conclusions.

If the final frequency of Curly in cage 3 is compared with the expected values at the end of 8 and 10 generations, the frequency actually obtained is significantly lower for both cases, with χ^2 values of 39.28 and 10.47 respectively (d.f. = 1); the χ^2 for significance at the 1 percent level is 6.635. Thus, in addition to the component of selection which results from the inviability of the Curly homozygotes, there is selection against the Curly heterozygotes as well.

At the termination of the experiment, the populations in cages 2 and 4 carrying the translocation were similar, as determined by a homogeneity test (χ^2 = 1.12, d.f. = 1, P = .20 − .30), and the data may be conveniently lumped. 131 out of 12,657 flies were Curly heterozygotes, giving a gametic frequency of 0.0052. However, the expected frequencies of Curly after 8 and 10 generations are lower, 0.0015 and 0.0004, respectively. Because of the size of the population, this difference is extremely significant; assuming that 8 generations had been completed, the χ^2 value for the difference is 228.31 (d.f. = 1). If as many as 10 generations had been completed, the χ^2 would be greater, viz. 1465.27 (d.f. = 1). Since this difference could scarcely be due to chance, we attribute it to the selective value of the Curly translocation apart from that resulting from the Curly homozygote lethality or the formation of inviable zygotic combinations from aneuploid translocation gametes. However, the difference between the expected and the actual results would be less if the percentage of orthoploid gametes formed were greater than 50%; PIPKIN (1940) studied a translocation that formed 59% orthoploid gametes.

The persistence of the translocation in the population may be due to the association of Curly with the translocation. If the depression of the adaptive value of Curly heterozygotes in the control cages is real, then somehow this effect is not manifested when Curly is associated with the translocation. An alternative explanation would be that the Curly heterozygote had itself undergone an improvement in reproductive fitness like that seen in the later generations of the experiment where successive generations of Curly were isolated. Or again, the translocation itself might be the cause of the better than expected viability if fewer aneuploid gametes than expected were produced.

It seems most likely, however, that Curly undergoes a favorable interaction with

the translocation, resulting in a higher adaptive value for the combination. It is well known that the combination of mutants which are individually deleterious, may result in superior viability under certain conditions, when compared to a standard; this has been demonstrated in *D. funebris* by TIMOFÉEFF-RESSOVSKY (1934), and by a number of workers in *D. melanogaster*.

SUMMARY

Using population cages consisting of a battery of six quart-size square milk bottles interconnected by a common central chamber, populations of *Drosophila melanogaster* of approximately 6000 individuals can be supported.

Studies were made of the adaptive value of Curly, a second chromosome dominant, lethal when homozygous, and associated with inversions in both arms of the chromosome, when Curly is in competition with its wild type homologue. Duplicate populations were maintained for a period of four months, during which time the frequency of the Curly chromosome dropped from an initial 25% to 3.12% in one population and to 6.05% in the other. It was shown that the drop was significantly more than would be expected on the basis of the lethality of the Curly homozygotes, and that selection must in addition act against the Curly heterozygotes to lower further the frequency of the Curly gene.

In an experiment with smaller populations, Curly was allowed to compete with its wild type homologue in successive, isolated generations. The frequency of Curly followed the curve of expectation through 13 generations, at which time it showed an improvement in adaptive value which persisted through the 30th generation; thus under certain conditions the relative adaptive value of Curly heterozygotes may improve when in competition with the wild type.

Population cage experiments of identical design were conducted to examine the adaptive value of Curly when associated with a 2,3 translocation. Again duplicate populations were maintained, and at the end of four months of selection, the gametic frequency of the Curly translocation had dropped from an initial frequency of 25% to 0.47% in one population and to 0.56% in the other. Although great, the drop in the frequency was not as great as would be expected on the basis of an additive effect of the lethality of Curly homozygotes and the inviability of zygotes formed from aneuploid translocation gametes. In fact, the frequency was maintained at a significantly higher level than was to be expected from inviability due to these causes alone, not to consider the lowering of viability that might be due to the Curly heterozygotes. It appears likely that Curly in association with the translocation had a favorable effect on the viability of the translocation heterozygotes.

ACKNOWLEDGMENTS

It is a pleasure to express my appreciation to PROFESSOR BENTLEY GLASS, who originally suggested the problem, and whose interest and guidance during the course of the work were invaluable. I am indebted also to DR. VERL L. HOUSE for his advice and criticism, and to MR. NELSON KRAUSE for his valuable suggestions and expert craftsmanship in the construction of the population cages and incubator.

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STUDIES ON THE POSITION EFFECT AT THE CUBITUS INTERRUPTUS LOCUS OF DROSOPHILA MELANOGASTER[1]

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WHEN DUBININ and SIDOROV discovered, in 1934, that translocations affecting the small fourth chromosome of *Drosophila melanogaster* frequently show a changed effect of the normal allele of the cubitus interruptus gene (ci^+), they posed the problem why some rearrangements gave this position effect and why others did not. Answers were provided in a series of papers by DUBININ, SOKOLOV, and TINIAKOV (1935), KHVOSTOVA and GAVRILOVA (1935), PANSHIN (1936, 1938) and particularly in the remarkably extensive work of KHVOSTOVA (1936, 1939). The following main conclusions in regard to chromosomes 2, 3, and 4 were reached:

(a) A necessary condition for a position effect of the *ci* locus is that the break in chromosome 4 lies between the kinetochore and the locus of *ci* (that is "proximal" to *ci*).

(b) Only those breaks give a position effect which transfer the distal part of the right arm of chromosome 4 to regions of chromosome 2 or 3 distal to the proximal first fifth or quarter of the chromosome arm involved in the translocation.

These investigations involved fourth chromosomes in which a *normal* allele of *ci* had been present at the time the rearrangements were produced. Later, SIDOROV (1941) and STERN and HEIDENTHAL (1944) reported on position effects of the *mutant ci* allele. On the basis of evidence from selected rearrangements SIDOROV concluded that the relations regarding the point of breakage in chromosomes 2, 3, and 4, and the existence or nonexistence of a position effect which had been found for ci^+ rearrangements ("$R(+)$") held also for rearrangements involving *ci* ("$R(ci)$"). Our own unselected sample of $R(ci)$ stocks permitted us to detect position effects not accessible to SIDOROV. The present paper will provide data to reconsider the following questions.

(1) What types of rearrangements give a position effect at the *ci* locus?

(2) What relation, if any, exists between the kind of rearrangement and the degree of position effect?

In addition experiments on the relative degrees of position effects of $R(ci)$ "position alleles" in heterozygotes with ci^+ and *ci* alleles, and the phenotypic effects of $R(ci)$ alleles in homozygotes, hemizygotes and compounds will be presented. Some new data on $R(+)$ position effects are also included.

The work covered in this report extended over a long period. The genetic tests were carried out first at the University of Rochester and later at the University of

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California by DRS. GERTRUDE HEIDENTHAL, H. D. STALKER, ELIZABETH WHITE SCHAEFFER, R. H. MACKNIGHT, M. KODANI, ALOHA HANNAH, C. STERN and MISS JOSEPHINE DE FRANK. Most of the cytological analyses are due to M. KODANI though H. D. STALKER had made a series of preliminary determinations and ALOHA HANNAH has, more recently, checked certain critical points. The names of all investigators could well have been listed as co-authors.

MATERIALS AND METHODS

The bases of investigation were 55 *R(ci)* rearrangements from irradiated *ci ey*^{R} males (*ey*^{R} = eyeless *R*, a recessive mutant in chromosome 4) and 19 *R*(+) rearrangements derived from X-rayed wild type Canton-S males. The *R*(+) rearrangements were detected by means of a *ci* position effect (STERN, SCHAEFFER, and HEIDENTHAL 1946). The *R(ci)* rearrangements were found, without reference to any possible position effect, by their giving linkage data characteristic of translocations involving chromosome 4 (STERN and HEIDENTHAL 1944).

All *R(ci)* alleles were originally derived from Y-bearing sperm. Among them one case of a Y-4 translocation was found, *R*^{1}*(ci)*, and two complex translocations, *R*^{12}*(ci)* and *R*^{40}*(ci)*, involving the Y chromosome simultaneously with 2-4 or 3-4 rearrangements (T(Y; 2; 4) and T(Y; 3; 4)). *R*^{1}*(ci)* will not be considered in this report. When the tests for position effects were made *R*^{40}*(ci)* had, presumably as a result of crossing over, become free of the Y-translocation but had retained a simple 3-4 translocation. A special rearrangement *R*^{24}*(ci)*, which involves a duplicate insertion of a section of 4 into 3, will not be treated in this paper. Finaly 10 *R(ci)* stocks were lost before completion of both cytological and genetic studies.

The *R*(+) alleles originally came from either X- or Y-sperm. Among the 17 *R*(+) alleles for which both genetic and cytological tests are available no X-4 or Y-4 rearrangement was present. Two stocks of *R*(+) alleles were lost before completion of the analysis.

The following genetically different types of chromosome 4 were used in various crosses: *ci*$^{+C}$, the wild type allele of *ci* present in the Canton-Special stock; *ci*$^{+2}$ *ey*^{2}, eyeless^{2}, recessive, and the wild type allele of *ci*$^{+2}$ present in the *ey*^{2} stock (STERN and SCHAEFFER 1943b); *ci*, cubitus interruptus; *ci ey*^{R}, cubitus interruptus, eyeless^{R}; *ey*^{D}, eyeless-dominant; *M-4*, Minute-4, a deficiency of several bands in region 101F, all of 102A and including 102B3; *M-4*^{5}, another deficiency, involving the right arm of chromosome 4 from close to the centromere to immediately after 102C4 (listed as *M-4*(+)*-15* by FUNG and STERN 1951). Apart from chromosome 4 which undergoes very little crossing over, all stocks used either in furnishing the rearrangements or in crosses had been made relatively isogenic with each other before the beginning of this work.

As reported in earlier papers the degree of interruption of veins was estimated by arbitrarily distinguishing five classes—0, 1, 2, 3, 4—from no interruption to complete absence of the section of the fourth vein located between the posterior cross vein and the wing margin (see fig. 1; STERN, MACKNIGHT, and KODANI 1946). A mean value of expressivity was then calculated by giving each class the weight of its class designation 0–4 and determining the mean of the distribution. Separate means

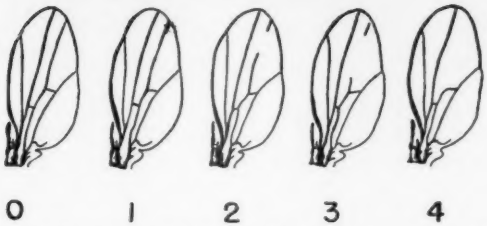

FIGURE 1. Representative samples of wings showing from no interruption (class 0) to complete absence (class 4) of the cubital vein.

were calculated for each culture, and each genotype and sex. In any one experiment total mean values for each genotype and sex were determined from the sum of the distributions of all cultures. This method of obtaining mean values of expressivity is admittedly crude. A better determination of means, particularly in cases of presumably truncated distributions is offered by transforming the data to inverse probability distributions. This has recently been done by HOUSE (1953) in a study of developmental interaction of mutants affecting venation. However, for the purposes of the present paper it seemed sufficient to employ the mean values of expressivity as derived in the simpler fashion. Various uncertainties are attached to the determination of mean expressivity of position effect alleles, as will be seen later. Therefore this account will be concerned with general trends rather than with specific values whose inherent uncertainties would be covered up by the pretense of exactness which is implied in the use of more refined statistical constants. In general the mean expressivity of any given genotype involving *R*— alleles will not be reported as such but rather in relation to a similar genotype without an *R*— allele. Thus, for instance, the mean expressivity value of *R(ci)/ci* individuals will be compared to that of *ci/ci* sibs and the *difference* between the two values, designated as expressivity index, entered in the tables. A positive difference indicates a higher expressivity of the *R*— genotype, a negative difference a lower expressivity.

Cytologically, rearrangements were analysed by means of permanent acetocarmine and aceto-orcein preparations of salivary gland chromosomes, and in reference to BRIDGES' map (1935a). Double bands were counted as units so that, for instance, the band 102B1 often referred to below actually corresponds to 102B1.2 of later maps (SLIZYNSKY 1944). For special purposes mitotic figures from neuroblasts and oogonia, were also studied.

THE LOCUS OF CI

The first data regarding the position of the *ci* locus within a specific region of chromosome 4 were provided by BRIDGES (1935b) by means of the *M-4* deficiency. *M-4/ci* flies exhibit an exaggerated *ci* phenotype. This pseudodominance of *ci* was interpreted to mean that the deficient region, from 101F to and including 102B3 had contained the *ci* locus (fig. 2). At the same time it was found by DUBININ, SOKOLOV, and TINIAKOV (1935) and confirmed by KHVOSTOVA and GAVRILOVA (1935), PANSHIN (1936), and KHVOSTOVA (1936) that a position effect of a normal allele of cubitus

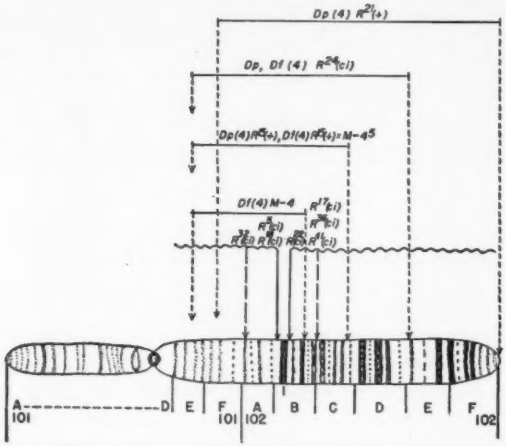

FIGURE 2. Map of salivary chromosome 4 of *D. melanogaster* combined after BRIDGES (1935 a, b) and SLIZYNSKI (1944) with locations of breakage points in various duplications, deficiencies and rearrangements. The two solid arrows indicate limiting breaks with heavy or weak position effects, respectively, of $R(ci)/+$.

interruptus appeared only when the break in chromosome 4 was in the immediate neighborhood of the centromere, or more specifically to the left of or within region 101F of the salivary chromosome map. From this it was concluded that the locus of *ci* lies to the right of 101F, and that a position effect is caused by the removal of the ci^+ allele from the heterochromatin in the centromere region.

Further evidence for localization of the *ci* locus in the right arm of the fourth chromosome is provided by three rearrangements in which the phenotypes of flies with the translocated part of chromosome 4 but without the remainder of the broken chromosome 4 showed the presence of the *ci* locus: (1) In $R^{21}(+)$, with breakage points in chromosome 2 just after 60E1 and in chromosome 4 in 101F, individuals deficient for the tip of 2 and having the translocated distal fragment of 4 in addition to a normal chromosome 4 with *ci* show a shift of venation toward normal thus indicating possession of the $R(+)$ allele in the fragment; (2) In R^{15} $(+)$ with an insertion into 30A of chromosome 2 of a section of chromosome 4 extending from immediately before 101F1 to immediately after 102C4, individuals which possess the insertion in addition to two cytologically normal chromosomes 4 with *ci* likewise show by a shift toward normal venation that the insertion contains the $R(+)$ allele; (3) Similarly, in $R^{24}(ci)$ the insertion into chromosome 3, just before 93F1 of a duplicated, inverse tandem section from close to the kinetochore of chromosome 4 up to and including 102D1 leads to a shift toward normal venation in flies possessing two cytologically normal chromosomes 4 with *ci*, thus placing the $R(ci)$ allele in the insertion. Furthermore, $R^{24}(ci)$ derivatives which have normal chromosomes 3, one *ci*-containing normal chromosome 4 and the deleted chromosome 4, show by their extreme, hemizygous-like vein interruption that the deleted chromosome does not contain the *ci* locus.

Indirect evidence on the localisation of the *ci* locus is based on the degree of position effects produced by breakages in different regions of chromosome 4. DUBININ and his collaborators observed position effect of *R*(+) alleles only when the break in 4 occurred in its right arm to the left of the *ci* locus. Cytologically, these breaks are close to the centromere, in section 101 or 102A. Thus, *ci* is located in the range 102B to 102C4. The cytology of our 17 *R*(+) alleles agrees with this conclusion. In 13 cases the break in 4 had taken place in the right arm of chromosome 4 in regions 101D–F, in three cases immediately before the band 102B1, and in one case in the left arm of 4.

It will be shown that position effects of *R*(*ci*) alleles occur not only with breaks to the left of 102B1 but also with breaks to the right of this band. However there is a striking difference in degree of effect according to whether the break lies to the left or the right of 10 2B1. In the 15 translocations involving the distal regions of chromosome 2 or 3 with a break in chromosome 4 to the left of 102B1 the index of expressivity of *R*(*ci*) alleles in combination with + (= *R*(*ci*)/+) ranged for females from .73 to 2.49, and for males from .85 to 2.62 (table 1). On the other hand the corresponding indexes for the 13 *R*(*ci*) alleles involving distal regions of chromosomes 2 or 3 with the fourth chromosome break to the right of 102B1 ranged for the females from 0 to only .14 and for the males from −.05 to only .20 (table 3).

Among the *R*(*ci*) alleles with a break to the left of 102B1, and with correspondingly high expressivity in *R*(*ci*)/+ there were three in which the break was located rather close to 102B1. In R^{32}(*ci*) it had occurred just before 102A1 and in both R^{5}(*ci*) and R^{18}(*ci*) it immediately preceded 102B1.

Among the *R*(*ci*) alleles with a break to the right of 102B1, and with correspondingly low expressivity in *R*(*ci*)/+ there were four in which the break was located rather close to 102B1. In three of the alleles, R^{17}(*ci*), R^{36}(*ci*) and R^{41}(*ci*) the break was just before 102C1, and in R^{22}(*ci*) it was immediately after 102B1. These facts narrow down the region for which a rearrangement to the left of it causes a strong position effect and one to the right of it a weak effect. Altogether the findings show the band 102B1 or its immediate neighborhood to be of crucial importance.

The classical interpretation of such results assigns the locus of *ci* to the band 102B1 or its immediate neighborhood. It has, however, been pointed out that the existence of a position effect may weaken the validity of a sharp definition of a locus (for a detailed discussion see GOLDSCHMIDT 1946). The data reported above, while showing effects of breaks occurring over a considerable length, are at least fully compatible with an assignment of the *ci* locus within or directly adjacent to the 102B1 band.

The question may be raised whether there is, typically, only one locus of *ci* or whether there may be a sequence of closely linked *ci* sites similar to the cases of pseudo-allelism discovered by E. B. LEWIS (1945), GREEN and GREEN (1949) and others. No data bearing on this question are available. At present the assumption of a single locus, at or close to band 102B1 appears sufficient.

THE EFFECTS OF R(CI) ALLELES HETEROZYGOUS WITH CI OR +

The position effect of the *R*(*ci*) alleles in heterozygotes was measured in two ways, by comparing the average vein interruption of *R*(*ci*)/*ci* with that of *ci*/*ci* flies,

and of $R(ci)/+$ with that of $ci/+$. Since various environmental agents affect the degree of interruption in *ci* and $R(ci)$ genotypes, the interruptions of $R(ci)/ci$ and $R(ci)/+$ flies were not compared with a standard value for ci/ci and $ci/+$ but with values obtained from sibs of the $R(ci)$ individuals. For this purpose two types of crosses were made with all $R(ci)$ alleles, usually *ci* ey^R ♀♀ × $R(ci)$ ey^+/ci ey^R ♂♂ and ci^{+2} ey^2 ♀♀ × $R(ci)$ ey^+/ci ey^R ♂♂. The F_1 of these crosses consisted of the two types of flies to be compared which had developed under essentially identical conditions. This method of comparison had the further advantage of largely cancelling the effects of possible modifiers of vein interruption which may have arisen in any one of the $R(ci)$ stocks.

On the other hand two possible sources of error are introduced in these crosses. One consists in the fact that some of the $R(ci)$ stocks contained individuals with two normal fourth chromosomes besides the $R(ci)$ translocation. This may lead to both diplo- and triplo-4 offspring. Values for degree of position effect derived from such mixed groups are not strictly comparable with values from diplo-4 flies only. This source of variation will be discussed in connection with selected, critical cases. For the majority of the results no detailed information on the diplo-4 or triplo-4 status of the flies is available. Presumably it accounts for a part of the variation in the observed values for position effects.

The second possible error in the determination of the effects of $R(ci)$ alleles lies in the fact that the $R(ci)$ individuals are non-eyeless (heterozygous for ey^R or ey^2) while their sibs are homozygous eyeless. However, homozygosity for ey^R or ey^2 has no appreciable influence on the expressivity of vein interruption. In crosses of *ci* ey^R ♀♀ × *ci* $+/ci$ ey^R ♂♂ the mean interruption in 390 *ci* $+/ci$ ey^R daughters was 2.65 as compared to 2.64 in 323 *ci* ey^R sisters, and 2.92 in 376 *ci* $+/ci$ ey^R sons as compared to 2.97 in 434 *ci* ey^R brothers. The differences beteeen the means for each sex, .01 and −.05, are not significant. Similarly, in two sets of crosses of ci^{+2} ey^2 ♀♀ × *ci* $+/ci$ ey^R ♂♂ (17°C) no significant differences in expressivity of *ci* $+/+$ ey^2 and *ci* $ey^R/+$ ey^2 sibs existed. In the first experiment mean vein interruptions in 2988 non-eyeless and 2463 eyeless females were .08 and .07, respectively, and, in 2793 non-eyeless and 2448 eyeless males, .13 and .11. The differences thus were .01 and .02. In the second experiment, 305 non-eyeless and 160 eyeless females had mean interruptions of .01 and .02, and 270 non-eyeless and 194 eyeless males .04 and .06. The differences, −.01 and −.02 are not significant. Since the eyeless genotype does not affect the expressivity of *ci*, references to the *ey* alleles will be omitted in the following except for table headings.

As seen in the foregoing, the expressivity of alleles at the *ci* locus is different in the two sexes. This is the reason why all data will be given separately for females and males. There is also a difference in the temperature at which various types of experiments were carried out. Low temperature increases the penetrance and mean expressivity of *ci* alleles. Therefore, low temperature was used in combinations in which penetrance at high temperature is close to zero. Unless otherwise noted all cultures were reared at ±26°C except those with $R(ci)+$ which were kept at ±17°C.

Translocations of ci to a distal region of chromosomes 2 or 3

Table 1 gives data on the 15 translocations in which a break in chromosome 4 had occurred to the left of band 102B1 and a break in chromosome 2 or 3 in any region except 37–44 and 77–85. These regions, located proximally on both sides of the centromeres 2 and 3 had been found by KHVOSTOVA (1939) not to produce position effects with *ci*. The translocations are listed in order of region to which the translocated part of 4 became attached. All translocations are simple reciprocals except $R^7(ci)$ in which the distal section of 4 is translocated to 24A1, of 2L, the tip of 2L substituted for the tip of 3R beyond 98A1 and the tip of 3R translocated to the base of 4. In addition the section 27B–29B is inverted.

These 15 $R(ci)$ alleles show highly significant position effects. $R(ci)/ci$ females are from .59 to 1.70 and $R(ci)/ci$ males from .22 to 1.63 class values more extreme than their like sexed sibs. Even more striking are the differences between $R(ci)/+$ and $ci/+$ flies, which range from .73 to 2.49 for females and from .85 to 2.62 for males. Since most $ci/+$ flies even at 17°C are normal—the mean expressivity being below .1—the indexes for $R(ci)/+$ flies in table 1 are an expression of the fact that usually the majority and sometimes all individuals show vein interruptions.

The variability of the degree of position effects in the different $R(ci)$ alleles will be discussed later.

TABLE 1

The effects of translocations of ci *to a distal region of chromosomes 2 or 3. F_1 of* ci ey^R ♀♀ × R(ci) +/ci ey^R ♂♂ *and* + ey^3 ♀♀ × R(ci) +/ci ey^R ♂♂

Number of $R(ci)$	Break in chromosomes**		Index $R(ci)$ +/*ci* ey^R		N†	Index $R(ci)$ +/+ ey^3		N‡
	4*	2 or 3	♀♀	♂♂		♀♀	♂♂	
7	101	2L i. b. 24A1	1.64	1.22	137	.73	1.07	127
53	101	2R i. a. 47B5	.92	.74	1760	1.47	1.60	389
33	101F	2R betw. 48C & D	.89	.80	333	1.58	1.73	281
3	101	2R i. b. 52E1	1.04	.79	118	1.40	1.49	317
55	101F	3L i. a. 65F2	.76	.64	1231	.99	1.13	275
5	i. b. 102B1	3L a. 69E2	.62	.22	456	.78	.85	374
8	101	3L a. 69F2	1.23	1.63	97	1.96	1.93	604
31	101	3L i. b. 75A1	.77	.87	260	2.26	2.46	293
52	101F	3L i. b. 76A1	1.70	1.28	1021	1.44	1.63	827
18	i. b. 102B1	3R a. 86C8	1.08	.94	1394	2.49	2.62	613
54	101F	3R 87F	1.29	1.09	435	—	—	—
23	101F	3R a. 92E2	.59	.29	1395	2.10	1.98	464
32	i. b. 102A	3R i. a. 92E2	1.04	.95	144	2.01	1.97	401
29	101	3R i. a. 94B2	1.34	1.04	1063	2.33	2.39	316
19	101	3R i. a. 97D9	1.26	.98	232	.92	1.12	279

* 101 signifies a break to the right of the centromere and to the left of region 101F.

** i. b., i. a. = immediately before or after . . .

† Sum of $R(ci)$ +/*ci* ey^R and *ci* ey^R/*ci* ey^R.

‡ Sum of $R(ci)$ +/+ ey^3 and *ci* ey^R/+ ey^3.

Translocations of ci to a proximal region of chromosomes 2 or 3

Among the translocations with a break to the left of 102B1 there were five which had their only other break close to, and six which had it within the chromocenter of chromosomes 2 or 3 (table 2). None had multiple breaks. In contrast to the preceding group of *R(ci)* alleles the position effects of the present group are much less striking or even absent.

Postponing briefly a consideration of $R^{9}(ci)$ and $R^{42}(ci)$ it is noted that the position effect indexes for the translocations with breaks slightly distal to the chromocenter of chromosomes 2 or 3 are smaller than the indexes for the translocations with breaks within the chromocenter. In the group with slightly distal breaks 15 values out of a total of 18 are within ±.15 of zero and thus are indicative of absence or near-absence of a position effect. The occurrence of two small negative values among the 15 may be regarded as a chance deviation from 0 or from a small intrinsically positive value. In contrast to the group with slightly distal breaks in chromosome 2 or 3 all 22 values in the group where the break was located directly in the chromocenter of 2 or 3 were larger than +.15, most of them considerably so.

$R^{9}(ci)$ showed very low position effect values in the $R^{9}(ci)/ci$ heterozygotes but relatively high values in $R^{9}(ci)/+$. The existence of a somewhat pronounced position effect for $R^{9}(ci)$ is not unexpected. The break point in chromosome 2, in 38B, is considerably more distant from the centromere than the break points of any other *R(ci)* alleles listed in table 2. Cytologically, $R^{9}(ci)$ thus approaches the *R(ci)* alleles of table 1 and the existence of some relatively high position effect values is the genetic counterpart of its cytological peculiarity.

The position effect values listed in the first of the two tests for $R^{42}(ci)$ are out of

TABLE 2

The effects of translocations of ci *to a proximal region of chromosomes 2 or 3. F_1 of crosses as in table 1*

Number of *R(ci)*	Break in chromosomes		Index *R(ci)* +/*ci* ey^R		N	Index *R(ci)* +/+ ey^3		N
	4	2 or 3	♀♀	♂♂		♀♀	♂♂	
9	101	2L a. 38B2	.17	.02	587	.47	.57	583
35	101	3L 79F	.02	−.01	465	.03	0	341
4	101	3L 80	−.12	.09	470	.02	.06	200
34	101	3L 80	.20	.17	1794	.08	.16	1726
34			—	—	—	.07	.15	420
25	101	3R 81F	.15	.06	1404	.11	.07	854
30	101	2 chrc.	.29	.17	357	.18	.20	360
42	101	2 chrc.	(−.16	−.10	946	.02	.01	621)
42			.48	.61	2780	.23	.37	1274
44	101	2 chrc.	.54	.61	489	.34	.58	1177
28	101	3 chrc.	.67	.63	679	.78	.66	282
37	101	3 chrc.	.45	.37	1395	.45	.52	3592
37			—	—	—	.28	.41	394
48	101	3 chrc.	.62	.50	1836	.33	.53	2261

line with the rest of the values for the $R(ci)$ alleles with a break within the chromocenter. The experiments which gave these $R^{42}(ci)$ values were carried out at the time when the main body of $R(ci)$ values were determined. More recently, when the unusual nature of the $R^{42}(ci)$ values was recognized, new experiments with $R^{42}(ci)$ were undertaken and the cytology of the stock re-investigated. The cytological nature of the translocation was confirmed but the genetic results given in the second row were clearly different from the earlier ones. The position effect values were now completely in line with those of the other $R(ci)$ alleles with a chromocentral break in 2 or 3. It is possible that an $R(ci)$ allele other than $R^{42}(ci)$ had mistakenly been used in the first experiment. The position effect values of only the more recent experiment will be considered in the later discussion.

Translocations of distal regions of chromosomes 2 or 3 to ci

In 13 translocations the break in 4 had occurred at various points to the right of 102B1, and the break in 2 or 3 anywhere outside the proximal regions 37–44 and 77–85. None involved 2L, six 2R, only one 3L and six 3R (table 3). All but two of these translocations are simple reciprocals in which the distal part of a long autosome

TABLE 3

Translocations of distal regions of chromosomes 2 or 3 to ci. *F_1 of crosses as in table 1*

Number of $R(ci)$	Break in chromosomes		Index $R(ci)$ +/*ci* ey^R		N	Index $R(ci)$ +/+ ey^+		N
	4	2 or 3	♀♀	♂♂		♀♀	♂♂	
16	a. 102D3	2R i. a. 45E1	−.23	−.05	305	0	−.04	352
16			−.10	−.08	1463	—	—	—
12*	102F	2R 46	—	−.30	544	—	0	144
36	i. b. 102C1	2R i. a. 47A1	−.22	−.19	2111	.02	.01	460
36			.02	−.13	176	—	—	—
36			−.12	−.02	2037	—	—	—
6	a. 102D3	2R a. 47D3	−.14	−.13	375	.01	.04	249
10†	i. a. 102C1	2R i. a. 48B1	−.23	−.29	1474	0	.01	256
45	a. 102 D–E	2R 58D	−.61	−.57	1906	.06	−.02	368
45			−.60	−.75	990	.07	.08	1237
45			−.40	−.48	5540	.14	−.05	966
17	i. b. 102C1	3L i. b. 67E1	.12	−.09	189	.03	.04	251
43	102F	3R i. b. 86A1	.10	.08	609	0	0	661
26	i. a. 102D1	3R i. a. 90B1	.31	.19	2982	.03	.02	1017
26			—	—	—	.06	−.01	809
40	102F2	3R i. b. 94A1	.73	.42	574	.13	.20	1143
41	i. b. 102C1	3R 98 C–D	.17	.15	2302	0	0	1983
22	i. a. 102B1	3R i. a. 98C1	.15	.81	998	0	.04	244
15	i. a. 102C1	3R i. a. 100A2	.37	.20	670	.05	.03	384

* Complex cytology: T(Y; 2; 4). No data on females. See text for calculation of $R(ci)$ +/*ci* ey^R index.

† Complex cytology.

had become attached to the base of 4 which had retained the *ci* allele. The two more complex translocations are $R^{10}(ci)$ and $R^{12}(ci)$. In $R^{10}(ci)$ six breaks have been established by observation, and a seventh, in the left arm of 4, is postulated. The established breaks are located (1) immediately after 48B1, (2) immediately before 50A1, (3) between centromere 3 and 81F, (4) in 85F (5) immediately after 97F1, and (6) immediately after 102C1. The new order is as follows: 2L, 2R to 48B1; 85F1–97F; 102C–102F. 3L, 3R to 81F; 50A1–60F. 4L tip; 81F–85F; 4L base, 4R to 102C1; 48B1–49F; 97F–100F. The most important part of this complex as far as it concerns the $R(ci)$ position effect is the chromosome which contains the centromere and the proximal regions of the original chromosome 4. Its left arm contains an intercalated proximal section of 3R and its right arm consists of the proximal part of 4, including the *ci* locus, followed by a short distal segment of 2R, and ending with the tip of 3R.

$R^{12}(ci)$ involves three clearly established and two postulated breaks. The former are all in 2R, at 46, 54B and 57B, the latter in 4 at 102F and in the Y chromosome. The rearrangement is as follows: 2L, 2R to 46; tip of 102F. 4L, 4R to 102F; 46–54B, 57B–60. Y with inserted 54B–57B. In regard to the new association involving the *ci* locus, $R^{12}(ci)$ may be described as a case in which the tip of 4 has been replaced by the right arm of 2R beginning at 46 but excluding a section from 54B to 57B. On account of the Y-linkage involved in this translocation usually no $R^{12}(ci)$ females are produced and, in crosses of $R^{12}(ci)/ci$ males to ci/ci or $+/+$ females, the ci/ci or $ci/+$ sons have duplications for the Y-inserted section of 2. Since the ci/ci males with the duplication are significantly more extreme in expressivity than normal ci/ci males, the position effect of $R^{12}(ci)/ci$ males has been measured not against the mean value of their sibs but against that of the nearly 10,000 ci/ci male sibs of other $R(ci)/ci$ flies.

Beginning with a discussion of $R(ci)/ci$ heterozygotes with the T(2R distal; 4 distal) $R(ci)$ alleles, each of the 11 initial indexes for the first tests are negative, that is, the mean degree of vein-interruption in the position allele heterozygote is less than that of *ci* flies with normally arranged chromosomes. One or more recent repeat experiments for three of the alleles, $R^{16}(ci)$, $R^{36}(ci)$ and $R^{45}(ci)$ added 10 more values

TABLE 4

Numbers of cultures for six T(2R *distal; 4 distal*) R(ci) *alleles which gave positive, negative and zero position effect indexes for* R(ci)/ci

Number of $R(ci)$	Index ♀♀			Index ♂♂		
	+	−	0	+	−	0
6	1	2	0	2	1	0
10	2	7	0	0	8	1
12	—	—	—	0	6	0
16	4	8	0	5	7	0
36	9	16	0	8	16	1
45	2	56	1	1	58	0
Total	18	89	1	16	96	2

which are listed in the second and third entries for these alleles. Nine of the indexes were again negative, one was positive, but very small (.02). Most of the $R(ci)/ci$ values deviated from ci/ci considerably less than one third of a class interval, but the six values for $R^{45}(ci)/ci$ were from .40 to .75 of a class interval more normal than those of their ci/ci sibs. While some of the negative values do not differ significantly from 0, on the whole the shift toward normality of venation in the six T(2R distal; 4 distal) $R(ci)$ alleles in $R(ci)/ci$ as compared to ci/ci flies is significant. This is reflected in table 4 which gives the number of individual cultures with positive, negative, and 0 differences. Out of the 222 indexes 185 are negative.

The single $R(ci)$ allele with one break to the right of ci and another in 3L, $R^{17}(ci)$, gave a small positive index for $R(ci)/ci$ females and a small negative index for males. These values do not differ significantly from zero, possibly because the numbers of individuals studied were small.

All 12 values for the differences between $R(ci)/ci$ and ci/ci sibs of the six $R(ci)$ alleles with one break to the right of ci and another in 3R were positive but relatively small.

All differences between $R(ci)/+$ and $ci/+$ of the present series of $R(ci)$ alleles are zero or very small. Only four values are negative. It might have been expected that the $R(ci)$ alleles with negative position effects in $R(ci)/ci$ heterozygotes would likewise give negative effects in $R(ci)/+$ heterozygotes. The $R(ci)/+$ experiments, however, were not suitable to revealing such possible effects. Since the mean expressivity for $ci/+$ in any experiment is often zero and since a mean of 0 signifies normality of venation which forms a phenotypic endpoint of the range of expressivity, $R(ci)/+$ flies even if intrinsically more normal than $ci/+$ flies cannot yield a still lower value. Even in experiments in which some $ci/+$ flies show vein interruptions the mean is so close to zero that no significant values for negative position effects can be obtained.

Translocations of regions including the proximal parts of chromosomes 2 or 3 to ci

In four of the $R(ci)$ rearrangements one break had occurred close to or within the chromocentric part of chromosomes 2 or 3 and the other in chromosome 4 to the right of 102B1. In these simple reciprocal translocations most or all of an arm of the long autosome had become attached to chromosome 4 distally to the ci locus. None of the position effects as measured in $R(ci)/ci$ flies was large (table 5). Seven values were

TABLE 5

Translocations of regions including the proximal parts of chromosomes 2 or 3 to ci. *F_1 of crosses as in table 1*

Number of $R(ci)$	Break in chromosomes		Index $R(ci)$ +/ci ey^R		N	Index $R(ci)$ +/+ ey^2		N
	4	2 or 3	♀♀	♂♂		♀♀	♂♂	
2	a. 102C3	2R i. a. 44C3	.32	−.11	199	0	.02	326
27	i. b. 102F1	3R i. a. 84B3, 4	.28	.18	1669	.02	.14	824
39	i. b. 102D1	3 chrc.	.24	.12	2870	.06	.06	1636
39			—	—	—	.07	.15	483
13	i. b. 102E1	3 chrc.	.01	.10	709	.03	.08	1078

positive, some of which deviated significantly from 0. One negative value did not differ significantly from 0.

All values for position effects of the $R(ci)/+$ combinations were zero, or positive and very small.

Tests for triplo-4 genotypes

An important source of error in determining position effect indexes would be the presence of any appreciable number of triplo-4 individuals among the $R(ci)$ flies. If, for instance, in the experimental series ci/ci ♀♀ × $R(ci)/ci$ ♂♂ some or all of the P males were actually $R(ci)/ci/ci$ then the F_1 would not consist solely of $R(ci)/ci$ and ci/ci flies but would also contain $R(ci)/ci/ci$ and $ci/ci/ci$ flies. Correspondingly in matings of $+/+$ ♀♀ × $R(ci)/ci/ci$ ♂♂ the F_1 would consist of four genotypes, namely $R(ci)/+$, $ci/+$, $R(ci)/ci/+$ and $ci/ci/+$. Dosage and interference effects (STERN 1943, 1948) would shift the phenotype of $ci/ci/ci$ toward normal as compared with ci/ci, and of $ci/ci/+$ toward greater abnormality as compared with $ci/+$. Similar shifts, in one or the other direction, would be expected in $R(ci)/ci/ci$ vs. $R(ci)/ci$ and in $R(ci)/ci/+$ vs. $R(ci)/+$ flies.

Triplo-4 individuals do indeed occur in $R(ci)$ stocks as proven by cytologic and genetic tests. It is, therefore, necessary to consider in some detail the consequences of their presence on the position effect indexes reported in tables 1, 2, 3, and 5.

1. ci/ci ♀ × $R(ci)/ci$ ♂ or $R(ci)/ci/ci$ ♂

(a) If the $R(ci)$ allele did not exhibit a position effect then $R(ci)/ci/ci$ flies would be phenotypically equivalent to $ci/ci/ci$ and $R(ci)/ci$ to ci/ci. A diplo-4 $R(ci)/ci$ P-♂ might produce some $R(ci)/ci/ci$ offspring besides the regular $R(ci)/ci$. Since, barring a position effect, the former would be more normal in venation than the latter, the sum of these $R(ci)$ flies would average more normal than their ci/ci sibs. Therefore, a *negative* value would be obtained for the mean difference between $R(ci)$ and ci flies.

(b) A *negative* value would also be expected if the P ♂ itself had been triplo-4 $R(ci)/ci/ci$. In this case, the majority of the $R(ci)$ offspring would be $R(ci)/ci/ci$ and the rest $R(ci)/ci$, and the majority of the ci offspring ci/ci and the rest $ci/ci/ci$. Therefore, again barring a position effect, the sum of the $R(ci)$ flies would average closer to normality than the sum of the ci flies.

2. $+/+$ ♀ × $R(ci)/ci$ ♂ or $R(ci)/ci/ci$ ♂

(a) If the $R(ci)$ allele did not exhibit a position effect and the P ♂ was diplo-4 $R(ci)/ci$ the sum of the regular $R(ci)/+$ and of triplo-4 $R(ci)/ci/+$ F_1 would be less normal than that of the $ci/+$ sibs. Thus, a *positive* difference between the averages of $R(ci)$ and ci flies would be observed.

(b) A *positive* value would also be found if the P ♂ had been $R(ci)/ci/ci$. In this case, the majority of the $R(ci)$ offspring would be $R(ci)/ci/+$ and the rest $R(ci)/+$ and the majority of the other sibs $ci/+$ and the rest $ci/ci/+$.

When these expectations, based on absence of position effects are compared with the results reported in tables 1, 2, 3 and 5, it is seen that the great majority of position effect values for the offspring of ci/ci × $R(ci)$ crosses are positive, in contrast to an

expectation, based on the presence of triplo-4 flies, of negative values. All these positive differences are therefore truly expressions of position effects although the specific indexes sometimes may possibly be compounded of a typical position effect value and a superimposed triplo-4 value. Besides the positive means, however, some negative ones are also listed. These will be the subject of further discussion.

For the offspring of $+/+ \times R(ci)$ crosses the occurrence of triplo-4 individuals even in the absence of a position effect would yield positive values. Actually such values were found nearly exclusively, the few exceptions giving zero values or small negative values essentially not differing from zero. However, many of the positive values, including all those listed in table 1 are much larger than accountable by the presence of triplo-4 flies. In earlier work, (STERN 1943) with flies raised at 13°C the mean expressivity of $ci/ci/+$ males was found to be only .66 class intervals greater than that of $ci/+$. Most likely the difference would be considerably smaller with flies raised at 17°C, the temperature employed in the present experiments. It is concluded, therefore, that all values in table 1 are true expressions of position effects.

This conclusion is in line with the earlier one according to which the positive values for $R(ci)/ci$ phenotypes definitely indicate position effects, since it is reasonable to assume that an $R(ci)$ allele which gives a position effect with *ci* also shows the effect in combination with +. The same consideration also applies to many of the smaller positive values of $R(ci)/+$ phenotypes, particularly those in table 2. These positive values may likewise be regarded as due to position effects.

Direct proof for position effect underlying both negative $R(ci)/ci$ and the relatively small positive $R(ci)/+$ values was finally obtained in a series of special tests. Here, the $R(ci)/ci$ P males were simultaneously crossed to ci/ci or $+/+$ females and to $M\text{-}4/ey^D$ females. If the P male was triplo-4, the $M\text{-}4/ey^D$ females would produce non-ey^D non-Minute ($M\text{-}4/+/+$) offspring besides the expected ey^D and *M* offspring of diplo-4 males. Only cultures from proven diplo-4 males and ci/ci or $+/+$ females were scored and their position effect indexes entered in the tables as stated below. In addition, cytological preparations of some of the female offspring were made.

The following $R(ci)$ alleles were thus tested (P ♂♂ by progeny tests, F_1 ♀♀ cytologically):

Table 2, $R^{34}(ci)/+$ (second line). All progeny tested 12 P males were diplo-4, as well as all 15 of their cytologically studied $R(ci)/+$ offspring.

Table 2, $R^{42}(ci)/ci$ (second line). All 11 P males were diplo-4.

Table 2, $R^{37}(ci)/+$ (second line). All eight P males and 31 of their $R(ci)/+$ F_1 were diplo-4.

Table 3, $R^{16}(ci)/ci$ (second line). All nine P males and 18 of their $R(ci)/ci$ offspring selected from the phenotypic class 1 were diplo-4. (The selection of the nearly normal class 1 $R(ci)/ci$ flies from the—on the average—less normal progeny should have favored the discovery of possible triplo-4 individuals if these had been responsible for the negative index.)

Table 3, $R^{36}(ci)/ci$ (second and third lines). All 14 P males were diplo-4.

Table 3, $R^{45}(ci)/+$ (second line). All nine P males and 25 of their $R(ci)/+$ F_1 were diplo-4.

Table 3, $R^{45}(ci)/ci$ (third line). Three class 0 and seven class 1 F_1 were diplo-4.

Table 3, $R^{26}(ci)/+$ (second line). All nine P males and 31 of their $R(ci)/+$ F_1 were diplo-4.

Table 3, $R^{40}(ci)/+$. Three class 1 $R(ci)/+$ F_1 were (probably) diplo-4. (The selection of class 1 or 2 $R^{40}(ci)/+$ and $R^{39}(ci)/+$ flies from the otherwise nearly exclusively normal progeny should have favored the discovery of possible triplo-4 individuals if these had been responsible for the positive indexes.)

Table 5, $R^{39}(ci)/+$ (second line). Four of eight P males were diplo-4, the other four triplo-4. Among 27 class 1 and 2 $R(ci)/+$ F_1 of the diplo-4 parents were 26 diplo-4 and one triplo-4.

These progeny tests and cytological determinations establish for the cases studied, that negative values for $R(ci)/ci$ are not due to presence of triplo-4 flies, and that likewise even small positive values for $R(ci)/+$ are not due to presence of triplo-4 flies.

Tests for duplications

Some $R(ci)$ rearrangements produce viable individuals which in addition to two normal second and third chromosomes contain a duplication of the translocated tip of one of these chromosomes. Such flies are deficient for part of chromosome 4. Usually the duplication-carrying flies were not included in this report since they are phenotypically distinguishable from their sibs. However, this is not true for $R^{45}(ci)$ flies. Therefore special tests were made with this allele which had given the largest negative $R(ci)/ci$ indexes (table 3). In crosses of $ci/ci \times R^{45}(ci)/ci$, two phenotypically indistinguishable $R(ci)$ genotypes occur among the offspring, regular translocation heterozygotes and duplication-2, deficiency-4 (*Dp* 2, *Df* 4) flies. In addition normal ci/ci and lethal *Df* 2, *Dp* 4 zygotes are produced. Thus the theoretical ratio of $R(ci)$ to ci flies is 2:1. On the other hand, the expected ratio of these phenotypes in crosses of $ci/ci \times R^{45}(ci)$ *Dp* 2, *Df* 4 is 1:1.

It was investigated whether the *Dp* 2, *Df* 4 individuals were more normal than regular $R(ci)/ci$ flies, since such a phenomenon could account for the observed negative expressivity values. F_1 males with $R(ci)$ phenotypes from $ci/ci \times$ regular $R^{45}(ci)/ci$ were classified according to degree of vein interruption and their progeny tested with ci/ci females. In their offspring either 2:1 or 1:1 ratios of $R(ci):ci$ were approximated. This permitted classification of the tested males into regular and *Dp* 2, *Df* 4 genotypes. The results (table 6) show that the majority of 44 fertile males were of regular genotype regardless of the degree of interruption. If the 13 sterile

TABLE 6

Tests for Dp 2, Df 4 *in* F_1 R(ci) *males from* ci/ci $\times$ R^{45}(ci)/ci

Genotype of tested male	Phenotypic class		
	1	2	3
Regular	4	24 (+1?)	9 (+1?)
Dp 2, *Df* 4	0	1 (+2?)	2
Sterile	0	6	7

males consisted of regular and *Dp* 2, *Df* 4 genotypes in the same ratio as the fertile males, then no significant difference in vein interruption would be caused by presence of the duplication as compared to regular *R(ci)/ci*. Should, on the other hand, the sterile males be mostly of *Dp* 2, *Df* 4 genotype, then this genotype would tend to give more—and not less—extreme interruption than the regular genotype. In either case these tests establish that the negative index for $R^{45}(ci)/ci$ is not due to the presence of *Dp* 2, *Df* 4 individuals. It is therefore the expression of a true position effect.

THE EFFECTS OF R(+) ALLELES HETEROZYGOUS WITH CI

Sixteen of the 17 *R*(+) alleles all of which were detected by means of their position effect followed the rules established by DUBININ, KHOVOSTOVA and their colleagues. In them the breakage points in chromosome 4 were located in the right arm, between the centromere and the locus of *ci*, and the breaks in chromosomes 2 or 3 in the euchromatic parts distal to the proximal fifth or fourth of the chromosome arm. The exception from the rule, $R^{2}(+)$, had one of its breaks in the left arm of chromosome 4 and the other in region 82D of 3R, close to the centromere of chromosome 3. All *R*(+) translocations were simple reciprocals except $R^{3}(+)$, $R^{14}(+)$ and $R^{15}(+)$. For a description of $R^{3}(+)$ and $R^{15}(+)$ the reader is referred to STERN, MACKNIGHT and KODANI (1946). In $R^{14}(+)$ three breaks are involved: (1) in the middle of 60B of 2R, (2) just before 99B1 of 3R, and (3) in 101F. The resulting rearrangement is as follows: 2L, 2R to 60B; 99B1 to 100. 3L, 3R to i.b. 99B1; 101F to 102. 4L, base of 4R to 101F; 60B to F.

TABLE 7

The effects of 17 R(+) *alleles.* F_1 *of* ci ey^R ♀♀ × R(+) +/ci ey^R ♂♂

Number of *R(ci)*	Break in chromosomes		Adjusted expressivity *R(+)/ci*		N
	4	2 or 3	♀♀	♂♂	
3*	101	2L a. 21D1	3.16†	3.32†	932
10	i. b. 102B1	2L b. 31F1	1.39	1.52	656
15*	101, 102C	2L 30A	3.08†	2.91†	552
11	i. b. 102B1	2R 47C	0.51	0.70	766
20	101	2R i. a. 55A	1.05	1.44	681
18	101F	2R i.b. 57F1	1.93	2.12	904
6	101	2R i. a. 59D	1.46	1.70	774
21	101F	2R i. a. 60E1	2.11	2.29	676
19	i. b. 102B1	3L b. 62D3	1.27	1.73	593
13	i. b. 102A	3L i. a. 65D1	2.33	2.43	770
2	left arm	3R 82D	3.03	3.26	1089
16	101F	3R a. 90E1	2.01	1.77	928
5	101F	3R i. a. 96E1	1.38	1.68	498
8	i. b. 102A	3R i. b. 96F6	1.42	1.90	673
9	101F	3R i. b. 97C1	2.43	2.57	492
12	101	3R i. b. 98F1	2.55	2.85	612
14*	101F	3R i. b. 99B1	1.54	1.70	685

* complex cytology

† see text

The mean values for the vein interruption of females and males of the different $R(+)/ci$ genotypes derived from crosses of $ci/ci \times R(+)/ci$ ranged from 1.03 to 3.44 and .95 to 3.46 respectively. These values are direct measures of the degree of position effects since typical heterozygotes, $+/ci$, raised at 26°C are normal, i. e. have a value of 0. The expressivity values for the $R(+)/ci$ flies are subject not only to intrinsic variability but also to secondary environmental or genetic modificaion. The existence of such factors is indicated by considerable correlation between the expressivity of the ci/ci female and male sibs of the different $R(+)/ci$ flies. The ci/ci values in 16 of the $R(+)$ series (exclusive of $R^{15}(+)$; see below) varied from 2.06 to 2.60 for females and from 2.34 to 2.97 for males. The over-all averages for 3786 ci/ci females and 3633 ci/ci males from these 16 series were 2.32 and 2.72. Since it is likely that the secondary modifiers of the expressivity of ci/ci also affect $R(+)/ci$ in a similar way, an approximate correction was made by adding to a given $R(+)/ci$ value (a), the difference between the average ci/ci value for all 16 experiments (b) and the ci/ci value of the given $R(+)$ experiment (c). The adjusted $R(+)/ci$ values, as entered in table 7, are thus equal to a + (b − c).

The adjusted values still are not corrected for secondary variability due to occurrence of triplo-4 or viable duplication individuals. For two of the $R(+)$ alleles, $R^{15}(+)$ and $R^{3}(+)$, it is known that such individuals are frequent (STERN, MACKNIGHT and KODANI 1946). In the case of $R^{15}(+)$ the $R(+)$ phenotypes are diplo-4 $R^{15}(+)/ci$ but half of the ci sibs contain an $R^{15}(+)$ duplication which makes them triplogenic $R^{15}(+)/ci/ci$. Therefore, the expressivity value of the $R^{15}(+)/ci$ flies could not be adjusted to that of its ci sibs but was entered directly in table 7. In $R^{3}(+)$ cultures the $R(+)$ phenotype is represented by genotypes $R^{3}(+)/ci$ and $R^{3}(+)/ci/ci$. The triplogenic flies are more nearly normal than the diplogenic ones. The adjusted $R^{3}(+)/ci$ values in table 7—though the highest in the whole series—are thus underestimates of the actual position effect of $R^{3}(+)$.

With the exception of $R^{2}(+)$, the results with $R(+)$ alleles agree with those of earlier workers. Some specific comments will be made in the discussion.

Comparisons of the degrees of R(ci) position effects in heterozygotes for ci and +

In a very general sense, a positive correlation exists between the degree of position effects for the different $R(ci)$ alleles in the combinations $R(ci)/ci$ and $R(ci)/+$. This is apparent from a survey of tables 1–3 and 5. The rearrangements with breaks to the left of the ci locus and in the distal regions of the long autosomes all give strong position effects in both types of heterozygotes (table 1). Most other rearrangements give weak effects, again in both types of heterozygotes (tables 2, 3, 5). Moreover, the series of rearrangements with breaks to the left of ci and in the chromocenter of the long autosomes (table 2, lower part) which as a group gives the relatively strongest position effects in $R(ci)/ci$ outside of those listed in table 1, also gives relatively strong effects in $R(ci)/+$.

In detail, however, the correlation in strength of position effects of $R(ci)$ alleles in heterozygotes with ci and + breaks down in numerous instances. Thus, in table 1, $R^{7}(ci)$ in combination with ci shows a greater position effect than $R^{53}(ci)$, while in in combinations with + the reverse is true. If the numbers of the 14 $R(ci)$ alleles of

table 1 for which both types of heterozygotes have been studied are listed in order of increasing strength of position effect in $R(ci)/ci$ female heterozygotes one obtains the following seriation: $23 < 5 < 55 < 31 < 33 < 53 < 32, < 3 < 18 < 8 < 19 < 29 < 7 < 52$. Their order in $R(ci)/+$ female heterozygotes is found to be $7 < 5 < 19 < 55 < 3 < 52 < 53 < 33 < 8 < 32 < 23 < 31 < 29 < 18$, thus showing a striking lack of correlation for the two combinations. Similarly, the ranking of the 14 $R(ci)/ci$ male heterozygotes in order of effect is as follows: $23 < 55 < 53 < 3 < 33 < 31 < 18 < 32 < 19 < 29 < 7 < 52 < 8$. The non correlated sequence for $R(ci)/+$ male heterozygotes is $5 < 7 < 19 < 55 < 3 < 53 < 52 < 33 < 8 < 32 < 23 < 29 < 31 < 18$. Many other examples of independence between the degrees of effect in the two types of heterozygotes can be seen by comparing different $R(ci)$ alleles listed in different tables or within the same table of the set of tables 1–3 and 5.

It is true that the data are not strictly comparable since the different types of heterozygotes were obtained at various times so that the results may have been affected by divergent environmental or genetic modifying conditions. It is unlikely, however, that such secondary variation is sufficient to account for the many striking reversals in the order of position effects in the $R(ci)/ci$ and $R(ci)/+$ heterozygotes. This has been proven specifically for two $R(ci)$ alleles selected from those listed in table 1, namely $R^{52}(ci)$ and $R^{18}(ci)$. At 26°C the $R^{52}(ci)/ci$ flies are nearly exclusively of the most extreme phenotype (average expressivity for females 3.98–4.00, for males 3.99) while $R^{18}(ci)/ci$ are less extreme (average expressivity ♀♀ = 3.17–3.32; ♂♂ = 3.60–3.70). In consequence, as seen in table 1, and copied in line 1 of table 8, the degree of position effect for $R^{52}(ci)/ci$ is greater than for $R^{18}(ci)/ci$ (differences highly significant). In contrast to this, the degree of position effect of $R^{52}(ci)/+$ is strikingly smaller than of $R^{18}(ci)/+$, the latter showing the most extreme effect among all $R(ci)/+$ heterozygotes. In order to test the validity of these findings, the experiments were repeated by mating single $R^{52}(ci)/ci$ and $R^{18}(ci)/ci$ males simultaneously and separately to both ci/ci and $+/+$ females and raising the offspring of the different females in separate cultures. In this way secondary environmental and genetic variability was greatly reduced. The results are shown in line 2 of table 8. They are essentially the same as in the less controlled experiments: greater position effect of $R^{52}(ci)/ci$ than of $R^{18}(ci)/ci$ but smaller position effect of $R^{52}(ci)/+$ than of $R^{18}(ci)/+$. (In a third experiment (line 3, table 8) the same mating procedures were

TABLE 8

Comparisons of the degrees of position effects of R^{52} (ci) *and* R^{18} (ci). *F_1 of* ci ey^R ♀♀ × R(ci) +/ci ey^R ♂♂ (*F_1 raised at 26°C except in April 1948 when 17°C was used*) *and F_1 of* + ey^2 ♀♀ × R(ci) +/ci ey^R ♂♂ (*F_1 raised at 17°C*)

Date of Experiment	Index $R^{52}(ci)$ +/ci ey^R		N	Index $R^{18}(ci)$ +/ci ey^R		N	Index $R^{52}(ci)$ +/+ ey^2		N	Index $R^{18}(ci)$ +/+ ey^2		N
	♀♀	♂♂		♀♀	♂♂		♀♀	♂♂		♀♀	♂♂	
1944	1.70	1.28	1021	1.08	0.94	1394	1.44	1.63	827	2.49	2.62	613
Feb. 1948	1.78	1.38	497	1.02	1.21	230	0.91	0.92	139	2.83	2.50	40
April 1948	0.59	0.28	824	0.56	0.29	702	1.36	1.10	627	2.48	2.45	452

used as in the second, but both the $R(ci)/+$ and the $R(ci)/ci$ offspring were raised at 17°C. In this experiment again the $R^{52}(ci)/+$ values were smaller than the $R^{18}(ci)/+$ values. However, the $R(ci)/ci$ values have become nearly identical, due to the shifting at the low temperature of nearly all phenotypes into the most extreme class.)

THE EFFECTS OF R(CI) HOMOZYGOTES

A number of different $R(ci)$ alleles were obtained as homozygotes, either in pure stocks or, if poorly viable or fertile, in stocks of the parentage $R(ci)/ey^D \times R(ci)/ey^D$ (ey^D usually lethal in homozygotes). In order to compare the expressivity of $R(ci)/R(ci)$ with that of ci/ci under similar conditions, cultures were simultaneously stocked with fertilized P females which would produce non-ey^D $R(ci)/R(ci)$ homozygotes (and sometimes $R(ci)/ey^D$ flies), and fertilized $ci\ ey^R/ci\ ey^R$ P females which would produce solely eyeless ci/ci homozygotes. No P males were placed into the culture bottles.

The mean expressivities of the $R(ci)/R(ci)$ and ci/ci homozygotes were determined and the differences entered in columns 2 and 3 of table 9. For purposes of comparison the differences between the mean expressivity of heterozygous $R(ci)/ci$ flies and their ci/ci sibs are listed in columns 4 and 5. As a control, cultures were prepared in which fertilized females from ci/ci and $ci\ ey^R/ci\ ey^R$ stocks were simultaneously permitted to produce offspring. The first row of table 9 shows that the F_1 ci/ci females were by .11 expressivity units more normal than the F_1 $ci\ ey^R/ci\ ey^R$ females (negative difference!) while the F_1 ci/ci males were by .05 units less normal than the F_1 $ci\ ey^R/ci\ ey^R$ males (positive difference). Both differences are small and, considering the general variability of expressivity, indicate that ci/ci and $ci\ ey^R/ci\ ey^R$ flies are equivalent in

TABLE 9

The effects of R(ci) *homozygotes. See text for methods used. The indexes of* R(ci)/ci *heterozygotes have been copied from tables 1 and 2*

Number of $R(ci)$	Index $R(ci)/R(ci)$		Index $R(ci)/ci$		N*
	♀♀	♂♂	♀♀	♂♂	
ci control	−.11	.05	.01	−.05	561, 180
52	.08	.04	1.70	1.28	555, 86
18	−.84	−.46	1.08	.94	211, 1779
18†	—	−.97	1.08	.94	69, 114
29	−.75	−1.24	1.34	1.04	218, 272
29†	—	−2.03	1.34	1.04	138, —
54	−.23	−.73	1.29	1.09	1672, 77
44	0	−.05	.54	.61	922, 596
28	.53	.36	.67	.63	150, 536
37	−.17	−.18	.45	.37	534, 722
48	−.04	−.04	.62	.50	271, 1984

* The first of the two numbers given under N represents the *ci/ci* or *Rci/Rci*, the second the *ci ey*^{R}*/ci ey*^{R} individuals.

† See text.

expressivity. The differences between the expressivities of *ci* +/*ci ey*^{R} and *ci ey*^{R}/*ci ey*^{R} given in the last two entrees of line 1, .01 and −.05 have been discussed earlier (p. 348).

Postponing briefly a consideration of the repeat experiments with $R^{18}(ci)$ and $R^{29}(ci)$ listed in rows 4 and 6, the table contains data on $R^{52}(ci)$, $R^{18}(ci)$, $R^{29}(ci)$ and $R^{54}(ci)$ which have their breaks to the left of *ci* and in a distal region of chromosome 3 (see table 1) and $R^{44}(ci)$, $R^{28}(ci)$, $R^{37}(ci)$ and $R^{48}(ci)$ in which one of the breaks is in the chromocenter of either chromosome 2 or 3 (see table 2). The first named four $R(ci)$ alleles produced a strikingly more extreme expressivity than *ci*/*ci* when acting in $R(ci)/ci$ heterozygotes, and the last four $R(ci)$ alleles in combination with *ci* also gave clearly more pronounced expressivity than *ci*/*ci* even though less extreme than the first group. Surprisingly enough not a single one of the $R(ci)/R(ci)$ homozygotes deviated as much from *ci*/*ci* as the corresponding $R(ci)/ci$ heterozygotes. On the contrary 11 of the 16 expressivity differences were not even intermediate in expressivity between *ci*/*ci* and $R(ci)/ci$ (positive indexes) but more normal than *ci*/*ci* (negative indexes).

The finding of a more nearly normal expressivity of $R(ci)$ homozygotes as compared to $R(ci)/ci$ heterozygotes and, in specific instances, as compared even to *ci*/*ci* homozygotes was confirmed by two experiments of somewhat different design included in rows 4 and 6 of table 9. The first of these deals with $R^{18}(ci)$. A series of single pair matings was made of non-eyeless flies from the $R^{18}(ci)$ stock which contained non-eyeless $R^{18}(ci)/R^{18}(ci)$ and $R^{18}(ci)/ci\ ey^R$ and eyeless *ci ey*^{R}/*ci ey*^{R} flies. One hundred eighty-nine F_1 males were classified in regard to their expressivity and then progeny tested individually in matings to *ci ey*^{R}/*ci ey*^{R} females. Six cultures were sterile. Among the fertile males, 69 gave only non-eyeless offspring and thus were $R(ci)/R(ci)$ homozygotes and 114 gave both non-eyeless and eyeless offspring and thus were $R(ci)/ci\ ey^R$. The distributions of the males over the array of expressivity classes is shown in table 10. It is seen that the $R^{18}(ci)/R^{18}(ci)$ phenotypes were greatly shifted toward normal as compared to the $R^{18}(ci)/ci$ phenotypes. The mean expressivities were 1.96 and 2.93 respectively, yielding a difference of −.97 as entered in table 9.

The second special experiment is concerned with $R^{29}(ci)$. This position allele was available in a homozygous $R^{29}(ci)/R^{29}(ci)$ stock. A classification of 138 males showed that 74 were without vein interruption (class 0), 43 in class 1, 21 in class 2 and none in the extreme classes, 3 or 4. Since this result is so strange it was felt desirable to verify the $R^{29}(ci)/R^{29}(ci)$ genotype of the males; all were mated to *ci ey*^{R}/*ci ey*^{R}

TABLE 10

Phenotypic distributions of $R^{18}(ci)/R^{18}(ci)$ *and* $R^{18}(ci)/ci$ *males. Genotypes determined by progeny tests*

	0	1	2	3	4
$R^{18}(ci)/R^{18}(ci)$	0	20	34	13	2
$R^{18}(ci)/ci\ ey^R$	0	1	23	73	17
Sterile	0	0	3	3	0

females. One third of the cultures were sterile but every fertile male produced only non-eyeless offspring. The progeny of four males, two belonging to class 0, and one each to classes 1 and 2 were classified in detail. They were all similar in giving extreme $R^{29}(ci)/ci\ ey^R$ phenotypes with a mean expressivity based on 800 flies of 3.59 for females and 3.63 for males, thus comparing well with the values of 3.52 and 3.48 obtained earlier and used in the determination of the position effect indexes for $R^{29}(ci)/ci$ listed in table 1. The mean expressivity of the 138 homozygous $R^{29}(ci)$ males was .62. Using the value of 2.65 for the mean expressivity of $ci\ ey^R/ci\ ey^R$ males as determined from 9927 male sibs of the $R(ci)/ci$ flies listed in tables 1–3 and 5 the shift of $R^{29}(ci)/R^{29}(ci)$ homozygotes toward normality is expressed in the difference −2.03 (table 9).

It is conceivable that some of the $R(ci)$ homozygotes were triplo-4 and contained a translocation-free $ci\ ey^R$ chromosome. This would account for a shift toward normality. However, this explanation cannot apply to at least the especially striking results with $R^{29}(ci)$. If the $R^{29}(ci)/R^{29}(ci)$ flies had been triplo-4 they would, in crosses to $ci\ ey^R$, be expected to have produced both diplo-4 and triplo-4 offspring with a resulting bimodality in venation phenotypes. No such bimodality was observed. Furthermore, in three crosses of $R^{29}(ci)$ homozygotes to $M\text{-}4/ey^D$, reported in the following section, the presence of a third chromosome 4 would have been discovered by the finding of non-$M\text{-}4$, non-ey^D offspring. No such flies occurred.

THE EFFECT OF R(CI) IN HEMIZYGOTES

In order to study the expressivity of $R(ci)$ hemizygotes, females heterozygous for $ci\ ey^R$ and various $R(ci)$ alleles were mated to $M\text{-}4^5/+$ males. The $M\text{-}4^5$ progeny of

TABLE 11

Phenotypic distributions of R(ci) +/M-4^5 *and* ci ey^R/M-4^5 *flies. The four subdivisions of this table correspond to the four types of* R(ci) *alleles listed in tables 1, 2, 3, and 5*

Number of $R(ci)$	$M\text{-}4^5/+^{ey}$										$M\text{-}4^5\ ey^R$			
	♀♀					♂♂					♀♀		♂♂	
	0	1	2	3	4	0	1	2	3	4	3	4	3	4
7	4	0	0	0	0	11	0	0	0	0	0	0	0	0
3	0	0	0	0	0	1	0	0	0	0	0	0	0	1
5	5	0	0	0	0	2	0	0	0	0	0	0	0	1
18	4	0	0	0	0	14	0	0	0	0	0	0	0	4
8	6	0	0	0	0	7	0	0	0	0	0	0	0	0
32	0	0	0	0	0	1	0	0	0	0	0	0	0	0
9	2	0	0	0	0	1	0	0	0	0	0	0	0	0
42	6	0	0	0	6	1	0	0	0	2	0	0	0	0
28	42	0	0	0	2	39	1	0	0	5	0	3	0	13
37	32	0	0	0	4	53	0	0	2	17	0	3	2	10
43	14	0	0	0	0	21	0	0	0	1	0	0	0	5
26	5	0	0	0	0	4	0	0	0	0	0	0	0	0
27	6	0	0	0	0	17	1	0	0	0	0	0	4	7
39	33	0	0	1	3	35	0	0	3	9	0	1	1	4

TABLE 12

Phenotypic distributions of R(ci) +/M-4 *and* ci ey^R/M-4 *flies*

Number of *R(ci)*	*R(ci)/M-4*										*ci ey^R/M-4*									
	♀♀					♂♂					♀♀					♂♂				
	0	1	2	3	4	0	1	2	3	4	0	1	2	3	4	0	1	2	3	4
44	0	0	0	0	0	2	0	1	2	2	0	0	0	0	0	0	0	0	0	0
48	0	0	1	11	2	0	0	0	4	3	0	0	0	0	0	0	0	0	0	0
29	32	0	0	0	0	112	14	1	0	0	0	0	0	0	0	0	0	0	0	0
ci ey^R	0	0	0	0	0	0	0	0	0	0	2	9	62	303	49	1	1	36	310	108

such crosses should consist of two types, *R(ci)/M-4^5* and *ci ey^R/M-4^5*. Since *M-4^5* is a deficiency for both *ci* and *ey*, the first type is expected to have large non-eyeless, the second the smaller "eyeless" eyes. It was found that eyeless in hemizygous state often is poorly expressed, the eyes being relatively large. This fact introduces uncertainties of classification. The viability of both types is very low.

The successful crosses, in which *R(ci)/M-4^5* and, usually, also *ci ey^R/M-4^5* offspring were obtained, include *R(ci)* examples of all four main groups of translocation alleles (see tables 1, 2, 3 and 5). The results are somewhat ambiguous (table 11). While all *ci ey^R/M-4^5* flies belong—as expected from earlier work with hemizygous *ci*—to the most extreme classes 3 and 4, the non-eyeless *M-4^5* flies fell into two phenotypic groups. In some *R(ci)* combinations all round-eyed *M-4^5* flies were completely normal in venation (class 0) or belonged to the nearly normal class 1. In others, the majority of the flies was normal while a minority belonged to the extreme classes 3 and 4. In the absence of a better explanation it may be assumed that the non-eyeless flies with extremely interrupted veins are normal eyed overlaps of the *ci ey^R/M-4^5* genotype and therefore actually belong to this genotype. If this interpretation is correct, then all *R(ci)/M-4^5* genotypes listed would give exclusively normal or nearly normal venation. This is very unexpected if one recalls the extreme expressivity of both hemizygous *ci* and often of *R(ci)/ci* heterozygotes. However, it falls in line with the shift toward normality of several of the *R(ci)* homozygotes.

Additional data on *R(ci)* hemizygotes were obtained from crosses of three *R(ci)* homozygotes to *M-4/+* or *M-4/ey^D* males (table 12). Altogether only seven *R^{44}(ci)/M-4* males were obtained, distributed over classes 0, 2, 3 and 4. Similarly 21 *R^{48}(ci)/M-4* flies were of the strongly interrupted classes 2, 3 and 4. Thus, they behaved similarly to the *ci/M-4* flies as obtained in a special control experiment (table 12 last line). On the other hand, *R^{29}(ci)/M-4* flies were mostly normal. Their expressivity values, 0 for females and .13 for males were considerably smaller than the values for *R^{29}(ci)/R^{29}(ci)* homozygotes from a simultaneous experiment reported in table 9 which were .52 for females and 1.27 for males (underlying the indexes −.75 and −1.24 as reported there).

THE EFFECTS OF R^x(CI)/R^y(CI) COMPOUNDS

Flies heterozygous for two different *R(ci)* alleles were obtained by mating homozygous *R^x(ci)/R^z(ci)* to *R^y(ci)/R^y(ci)* flies or, in a few cases to *R^y(ci)/ey^D*, where *R^z(ci)*

and $R^{y}(ci)$ signify two different $R(ci)$ alleles. In order to compare the expressivity of the $R^{z}(ci)/R^{y}(ci)$ compounds with that of ci/ci a similar method was used as in the experiments with $R(ci)$ homozygotes. It consisted of placing into the culture bottles fertilized females which would produce $R^{z}(ci)/R^{y}(ci)$ offspring, as well as $ci\ ey^{R}/ci\ ey^{R}$ females fertilized by males of the same genotype.

Three groups of $R^{z}(ci)/R^{y}(ci)$ compounds were studied, namely compounds (1) between $R(ci)$ alleles both of which had their breaks distally in a long autosome, (2) between one $R(ci)$ allele with a break distally and another $R(ci)$ allele with the break in the chromocenter of a long autosome, and (3) between $R(ci)$ alleles both of which had their breaks in the chromocenter of a long autosome (table 13). All $R(ci)$ alleles used had the break in chromosome 4 proximal to the *ci* locus.

Without exception the 42 female and male compounds were closer to normal than one or both of the constituent $R(ci)$ alleles when heterozygous with *ci*. Thus, in group (1) of compounds, involving $R^{52}(ci)$, $R^{18}(ci)$ and $R^{54}(ci)$, the expressivity differences between $R(ci)/ci$ and ci/ci varied between .94 and 1.70 (table 1) while those

TABLE 13

The effects of $R^{x}(ci)/R^{y}(ci)$ *compounds. See text for methods used*

Group	Numbers of $R(ci)$'s	Breaks in regions†	Index $R^{z}(ci)/R^{y}(ci)$		N*
			♀♀	♂♂	
(1)	52/18	76, 86	.19	.17	1349, 761
	52/54	76, 87	.39	.39	694, 951
	54/18	87, 86	.31	.38	1405, 752
(2)	52/44	76, chrc. 2	.67	.60	361, 963
	52/28	76, chrc. 3	.12	.18	971, 521
	52/37	76, chrc. 3	.26	.14	224, 981
	52/48	76, chrc. 3	−.11	−.10	142, 555
	18/44	86, chrc. 2	.61	.42	645, 792
	18/28	86, chrc. 3	.31	.34	620, 1074
	18/37	86, chrc. 3	.22	.45	468, 956
	18/48	86, chrc. 3	.10	.22	857, 818
	54/44	87, chrc. 2	.41	.33	759, 547
	54/28	87, chrc. 3	.31	.21	874, 541
	54/37	87, chrc. 3	.17	.32	805, 639
	54/48	87, chrc. 3	.45	.54	359, 537
(3)	44/28	chrc. 2, chrc. 3	.07	−.03	361, 630
	44/37	chrc. 2, chrc. 3	−.07	.05	631, 760
	44/48	chrc. 2, chrc. 3	.60	.40	336, 494
	28/37	chrc. 3, chrc. 3	−.07	−.36	664, 443
	28/48	chrc. 3, chrc. 3	−.07	.24	465, 315
	37/48	chrc. 3, chrc. 3	−.46	−.16	648, 433

* The first of the two numbers given under N represents the $R^{z}(ci)/R^{y}(ci)$, the second the $ci\ ey^{R}/ci\ ey^{R}$ individuals.

† The breaks listed refer to the long autosomes. All breaks in chromosome 4 were proximal to *ci*. For details consult tables 1 and 2.

between $R^{z}(ci)/R^{y}(ci)$ varied between .17 and .39 (table 13). In group (2) of compounds involving the $R(ci)$ alleles of the first group in combination with $R^{44}(ci)$, $R^{28}(ci)$, $R^{67}(ci)$ and $R^{48}(ci)$, the expressivity differences of the compounds ranged from $-.11$ to $+.67$ which is again below the minimum difference involving the first-named $R(ci)$ alleles. Moreover, the majority of the $R^{z}(ci)/R^{y}(ci)$ compounds in group 2 were also more normal than the $R^{44}(ci)$, $R^{28}(ci)$, etc. alleles in heterozygotes with *ci*. The differences between $R^{44}(ci)/ci$, etc. and ci/ci varied between .37 and .67 (table 2), while among the 24 differences between $R^{z}(ci)/R^{y}(ci)$ of the group (2) and ci/ci, 16 were below .37 (table 13). Finally, in group (3) of $R^{z}(ci)/R^{y}(ci)$ compounds 10 out of the 12 differences ranged between $-.46$ and $+.24$ and thus were smaller than the differences between ci/ci and any of the $R(ci)$ alleles in this group when heterozygous for *ci*. Seven of these 10 values were negative which corresponds to a phenotype more normal than that of ci/ci.

The shift toward low expressivity of $R^{z}(ci)/R^{y}(ci)$ compounds as compared to $R(ci)/ci$ heterozygotes containing the constituent $R^{z}(ci)$ or $R^{y}(ci)$ is alike in direction to that of $R(ci/R(ci)$ homozygotes. A comparison of the data in tables 9 and 13 shows that the shift toward normality for compounds, group (1), involving $R^{52}(ci)$, $R^{18}(ci)$ and $R^{54}(ci)$ is less striking than for the homozygotes of these alleles and that the same holds true for most, but not all, compounds of groups (2) and (3).

Differences of mean expressivity in females and males

The mean expressivity for ci/ci is lower in the female than in the male. In two independent series of experiments at 26°C, the difference between the values for the two sexes was found to be .32, and .42 respectively (table 14, b, f. See footnote to

TABLE 14

Differences of mean expressivity in females and males of different genotypes

	Numbers of diff. (♀ − ♂)			Mean diff. (♀ − ♂)*			
				positive		negative	
	pos	neg	0	mean	range	mean	range
(a) Means $R(+)/ci$	3	14	0	.20	.08–.36	.16	.01–.30
(b) Means $ci\ ey^{R}/ci\ ey^{R}$	0	17	0	.23	.13–.28	.32	.15–.49
(c) Indiv. cultures of (a) excl. those $R(+)$ with positive mean	12	58	3	—	—	—	—
(d) Indiv. cultures of (b)	4	82	0	—	—	—	—
(e) Means $R(ci)/ci\ ey^{R}$	2	41	1	.04	.03–.04	.33	.06–.63
(f) Means $ci\ ey^{R}/ci\ ey^{R}$ (sibs of e)	0	44	0	.42	.37–.47	.42	.19–.70
(g) Indiv. cultures of (e) excl. those $R(ci)$ with positive mean	12	242	1	—	—	—	—
(h) Indiv. cultures of (f)	8	265	0	—	—	—	—
(i) Means $R(ci)/+$ 17°C	6	32	4	.04	.01–.11	.11	.01–.34
(j) Means $ci/+$ 17°C (sibs of (i))	0	26	13	.02	0–.06	.03	.01–.09

* The mean differences (♀ − ♂) are given separately for cultures where the mean differences for $R(+)$ or $R(ci)$ sibs were positive and for those where they were negative. The combined mean difference (♀ − ♂) for ci/ci of line (b) was .32.

table.) In 86 individual cultures of the first series, only four and in 273 cultures of the second series, only eight had a higher value for females than for males (d, h).

R(ci)/ci flies show a similar differential with only two out of 44 differences of mean values and only 12 out of 255 cultures in favor of the females (e, g). The two positive differences out of 44 were very small, and the single case with equal expressivity for the two sexes was $R^{52}(ci)/ci$ in which nearly all flies belonged to the most extreme class 4, the mean expressivity for females and males being 3.99.

The situation is different for the *R(+)/ci* phenotypes (a, c). Among the 17 different genotypes three showed a higher expressivity for the females, and the same was true for 12 among 73 cultures. Moreover, the mean positive difference, in favor of the females, was relatively large, namely .20, and the mean negative relatively small, namely .16. It thus appears that the expressivity in the two sexes of the *R(+)/ci* types is shifted toward a positive value, as compared to *ci/ci* and *R(ci)/ci*. This shift seems not to be related to the mean degree of expressivity since most values for the three genotypes lie within the same range and since the *R(+)/ci* genotypes with positive and negative values for the difference in expressivity for the two sexes are randomly distributed over the range of mean expressivities.

There is a suggestion of a similar shift for the *R(ci)/+* values (i, j). A control series of 39 *ci/+* values contained no positive value (among the 13 values with no difference between the sexes there were 11 with expressivity 0, i.e. only normal flies, and two values with mean expressivities .004 and .01). In contrast, the 42 *R(ci)/+* values contained six positive ones.

The reason for the relatively increased expressivity primarily of the *R(+)/ci* combinations in the female sex is not clear. It seemed possible that the shift was due to secondary causes, such as greater frequency of triplo-4 flies in one than in the other sex. If, for instance, the P males frequently had been *R(+)/ci/ci*, and if preferential segregation had occurred such as to make (a) *R(+)* alone segregate more frequently with the X than with the Y chromosome and/or (b) *R(+)/ci* segregate less frequently with the X than the Y chromosome, then the *R(+)/ci* progeny would contain a surplus of females and/or the *R(+)/ci/ci* progeny a surplus of males. Since *R(+)/ci* is phenotypically more extreme than *R(+)/ci/ci* the difference in expressivity in the two sexes would be diminished or even reversed. However, this hypothetical mechanism of preferential segregation in supposedly triplo-4 P males would also change the difference in expressivity of the *ci* sibs. These would consist of *ci/ci* and *ci/ci/ci* flies. This consequence alone would result in a more normal mean expressivity for which, with one exception, there is no evidence. Moreover, the preferential segregations would result in a surplus of the complementary classes of *ci/ci/ci* sons over *ci/ci/ci* daughters, and of *ci/ci* daughters over *ci/ci* sons which would decrease the over-all difference in *ci* expressivity for the two sexes. This expectation is not borne out by the facts (table 14, b). The mean of the 14 values from *ci* sibs of *R(+)/ci* combinations with negative values does not differ significantly from the mean of the three values from *ci* sibs of *R(+)/ci* combinations with positive values.

If no secondary mechanism of the kind outlined can be found to explain the reduction or reversal of sexual difference in the expressivity of *R(+)* alleles in com-

bination with *ci* as contrasted with *ci* itself and, probably, with *R(ci)* alleles then the cause must lie in an intrinsically different reaction of *R(+)* alleles in development. No attempt will be made to speculate on the nature of such a different reaction.

DISCUSSION

LEWIS' (1950) penetrating review of the phenomenon of position effect contains a description and analysis of many facts and of the interpretations offered by various authors. In cases of the stable "S-type" position effects the concept of localized interactions between genes and gene products was elaborated into a fruitful theory. The variegated "V-type" position effects to which, with some uncertainty, the *ci* effect may be assigned, have not yet been amenable to a successful unifying treatment. While the present report adds new facts which permit the formulation of some rules no deeper insight into the mechanism of position effect at the *ci* locus can be presented.

The data on *R(ci)/ci* and *R(ci)/+* heterozygotes show that position effects at the *ci* locus occur not only with rearrangements involving a break between the centromere and the locus of *ci* but that breaks distal to the locus also result in position effects. Moreover, contrary to statements by earlier workers based on the results with selected *R(+)* alleles proximal as well as distal regions of chromosomes 2 and 3 change the effect of *ci*, though to different degrees, when brought in association with this locus. This had been foreseen by SIDOROV (1941) who expressed the opinion that "any break near the *c.i.* gene practically causes some change in its action". The validity of this statement has now been established by the study of an unselected series of *R(ci)* alleles.

No systematic search for heretofore undetected, presumably weak position effects involving *R(+)* has been made. In analogy with the findings on *R(ci)* it may be assumed that *R(+)* position effects are likewise produced not only by translocation of the ci^+ allele to distal breaks in chromosomes 2 and 3 but also by other types of rearrangements between chromosome 4 and with 2 or 3. There are a few data which support this assumption. Thus, SIDOROV (1941) studied two *R(+)* alleles with proximal breaks in chromosome 3 (and, presumably, proximal breaks in chromosome 4) which in the combination *R(+)/ci* did not produce position effects. The same *R(+)* alleles, however in the combinations *R(+)/R(ci)* produced noticeable expressivity shifts. Further indication of the production of *R(+)* position effects by rearrangements with chromosomes 2 or 3 other than translocation of ci^+ to distal regions is provided by the $R^2(+)$ allele. Here, the break in chromosome 4 was in the left arm so that the continuity of the *ci* locus with its neighboring material in the right arm was not disturbed. Nevertheless, the translocation of a distal section of chromosome 3 to the left arm, i.e. beyond the centromere, resulted in a position effect. An alternative interpretation of $R^2(+)$ is that a mutation of ci^+ to a *ci* allele was induced simultaneously with the rearrangement (LEWIS 1950).

Among the *R(ci)* alleles in which *ci* has become translocated to chromosome 2 or 3 (tables 1, 2) three different groups can be distinguished jointly by (a) their degrees of position effect in *R(ci)/ci* and *R(ci)/+*, and (b) the location of the break in chromosome 2 or 3. Group 1 gives usually striking effects, group 2 weak effects or ab-

sence of effect and group 3 intermediary effects. All these position effect indexes, with two probably insignificant exceptions, were positive. In group 1 the breaks are distal to the first proximal fifth or quarter of the chromosome arm involved. In group 2 the breaks are close to though still distal to the chromocentral region. In group 3 the breaks are located within the chromocenter. The single partial exception is $R^9(ci)$ listed in table 2, among group 2. While its $R^9(ci)/ci$ index is low as expected in group 2 its $R^9(ci)/+$ index is of intermediary strength. In reality, as pointed out earlier, for $R^9(ci)$ the break in chromosome 2 is more distal than all other relatively proximal breaks of the other alleles in group 2. $R^9(ci)$ thus forms a connecting link between groups 1 and 2 both as to degree of effect and location of break. The relation between degree of position effect and location of break in the long autosomes is illustrated for $R(ci)/ci$ females in figure 3 in which the abcissa represents chromosomes 2 and 3—superimposed upon another—from the left ends through the chromocenter to the right ends, and the ordinate the degree of position effect as measured

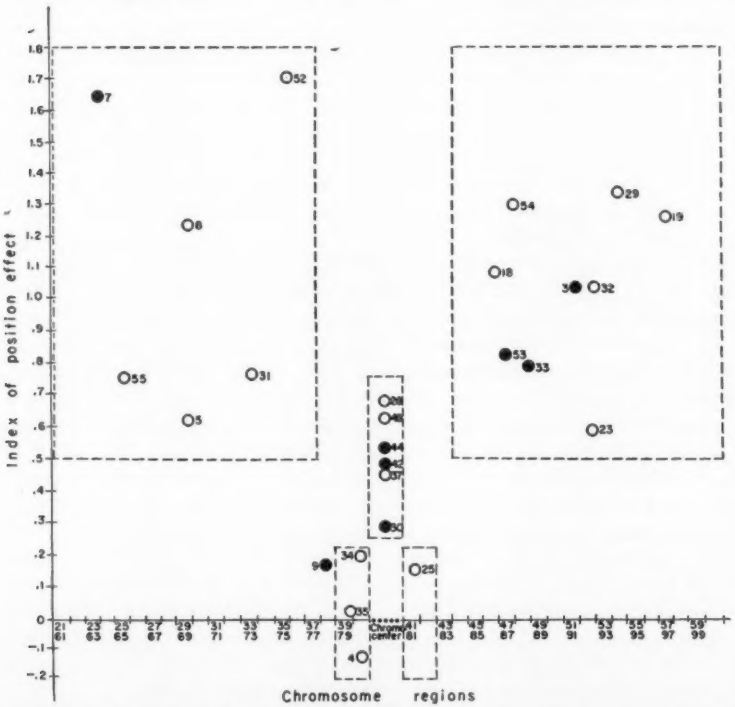

FIGURE 3. Position effects of $R(ci)/ci$ heterozygous females. Breaks in chromosome 4 between centromere and *ci*. Relation between location of the break in chromosome 2 or 3 and degree of position effect. The abscissa represents superimposed upon one another regions 21–60 of chromosome 2 and 61–100 of chromosome 3. The numbers designate the $R(ci)$ alleles. Solid circles = $R(ci)$ alleles involving chromosome 2. Open circles = $R(ci)$ alleles involving chromosome 3.

by the index. The same kind of diagrams with very similar results can be constructed from the data of tables 1 and 2 for *R(ci)/ci* males, and for *R(ci)/+* females, and males.

The locus of *ci*, in its normal position in chromosome 4, is distal to the centromeric region but still close to it. Indeed, the whole short chromosome 4 may be regarded as equivalent in reference to its neighborhood to the centromere to the proximal regions of chromosomes 2 and 3. It is noteworthy that the *R(ci)* alleles of group 2 which give the weakest position effects are the ones in which the *ci* locus has been translocated to a proximal region of chromosomes 2 or 3. Apparently, the new location of the translocated *ci* allele is physiologically similar enough to its normal location so as to result in no change or only a slight change in effect. In contrast to translocation into a proximal region, the *R(ci)* alleles of groups 1 and 3 which are transferred to either a distal or a chromocentric region show greater changes in their effects.

These facts may be interpreted in the sense that the normal locus of *ci* lies in a region which is similar to the heterochromatic sections adjacent to the centromeres of chromosomes 2 and 3. Striking position effects of the *ci* alleles thus result from their juxtaposition to euchromatic sections. In this respect the *ci* position effects resemble those of light (*lt*) in chromosome 2 (SCHULTZ 1936). Variegation caused by position effect at this locus is suppressed in the X0 male and enhanced in individuals with extra Y chromosomes. In regard to *ci* position effects, the role of the Y chromosome is not clear. KHVOSTOVA (1939) has stated that *R(+)* position effects "become(s) mostly more pronounced" in XXY females, but the author herself concludes that "the data are insufficient". On the other hand, ALTORFER (1951) has shown that some *R(ci)* position effects are enhanced in X0 males while others remain unchanged. The enhancement of *ci* effects contrasts with the suppression of light effects and thus is similar to the enhancement in regard to position alleles of euchromatic loci brought into heterochromatic neighborhoods.

Within each of the three groups of position alleles represented in figure 3 there is great variation of degree of position effects from one *R(ci)* allele to another, particularly in group 1. This variability may be due partially to secondary causes but much of it presumably depends upon the specific location of the various breakage points. No clear correlation between degree of effect of *R(ci)* alleles and location of presumed intercalary heterochromatic regions is apparent though HANNAH (1951) pointed out a relation between breaks associated with *R(+)* position effects and intercalary heterochromatin.

Had a correlation been found within each group between specific location of break and degree of position effect it could have related to part of the data only. This is so because there is largely independence between the degree of effect of the *R(ci)* alleles in heterozygotes with *ci* and the degree of the same *R(ci)* alleles in heterozygotes with +. This lack of correlation in effects points to qualitative differences in the developmental reactions of the different *R(ci)* alleles or their products. A suggestion of qualitative differences in the nature of *R(+)* alleles as compared with +, *ci* and *R(ci)* is also seen in the reduction in *R(+)/ci* of the difference of expressivity in the two sexes or even a reversal in direction of the difference.

Different *R(+)/ci* heterozygotes are equally variable in their position effects as

heterozygotes involving different *R(ci)* alleles. This variability has been discussed previously by DUBININ and SIDOROV (1934 a, b) and KHVOSTOVA (1939) and is borne out by the additional data of table 7 of the present paper. Similar to the $R^{9}(ci)$ allele which is associated with a break at 38B1 in chromosome 2 and which gave a smaller position effect index than the *R(ci)* alleles with more distal breaks, DUBININ and SIDOROV's *R*(+)-398, KHVOSTOVA's *R*(+)-III-IV/1, and our $R^{11}(+)$ allele have all relatively proximally located breaks in chromosomes 2 or 3 and, simultaneously, relatively weak position effects. For the more distally located *R*(+) alleles our data agree with KHVOSTOVA's who concluded that "a definite connection between the degree of expression of *ci* and the location of the breaks could not be established" (translated).

One of the unexpected findings concerns *R(ci)* alleles in which the break in chromosome 4 had occurred distal to the *ci* locus so that the tip of chromosome 4 became replaced by a section of chromosome 2 or 3 (tables 3, 5). It is not surprising in itself that position effects result from such translocations since position effects due to breakage distal to a locus had earlier been shown to be produced in *D. melanogaster* for curled (PANSHIN 1935), and position effects due to breakages on either side of a locus had been described for Notch (see HANNAH 1951) and, in *D. virilis*, for peach (BAKER 1953). It is not surprising either that the effects are usually weak since it could be assumed that the undisturbed relation between the chromocentral region of chromosome 4 and the *ci* locus would permit a relatively unchanged effect. This expectation was indeed fulfilled for 11 out of the 17 *R(ci)* alleles with distal breaks in chromosome 4. In ten out of 11 cases a section or most of the right arm of chromosome 3 had been translocated to chromosome 4 and in the eleventh case most of the right arm of chromosome 2 beginning with the proximal region 44C. The unexpected finding refers to the six *R(ci)* alleles in which a distal section of the right arm of chromosome 2, from as relatively proximal as 46 to as distal as 58D had been jointed to chromosome 4. In all of these six cases the *R(ci)/ci* phenotypes were shifted toward normal as compared to *ci/ci*, resulting in a negative position effect index. It thus seems as if the distal parts of the right arm of chromosome 2 are, in some essentially unknown respect, different from all tested sections of either arm of chromosome 3. (No comparison with the left arm of chromosome 2 is available).

Negative indexes are not restricted to the last named group of *R(ci)* alleles. They are found among *R(ci)/R(ci)* homozygotes, *R(ci)* hemizygotes, and $R^{z}(ci)/R^{y}(ci)$ compounds. Furthermore, the phenotypes of the tested homozygotes were all shifted toward normal as compared with *R(ci)/ci*, the phenotypes of some of the hemizygotes were completely normal, and those of all compounds were closer to normal than one or both of the constituent *R(ci)* alleles when heterozygous with *ci*. Since the mutant *ci* allele is less effective than normal ci^{+} alleles in leading to uninterrupted venation, the negative indexes signify a partial or complete restoration, in certain genotypes, of effectiveness of the *ci* allele in translocations. It may be mentioned that somewhat similar paradoxical situations concerning degrees of dominance have been discovered in the mouse (GRÜNEBERG 1953; older literature is cited in this publication). The gene *mi* for microphthalmia, in homozygotes, is much more strongly expressed than the allele Mi^{wh} in homozygotes. However, in the heterozygotes with the normal

allele *mi* has a much weaker effect than Mi^{wh}. Likewise, the expression of the gene *W* for macrocytic anemia is much more extreme than of the allele W^v in the homozygotes, but $W/+$ heterozygotes have a normal blood picture while $W^v/+$ mice are macrocytic.

The present data on homozygotes, hemizygotes and compounds include solely *R*(*ci*) alleles in which *ci* has been translocated to chromocentral or distal regions of chromosomes 2 or 3. It will be necessary in the future to fill various gaps in information particularly regarding the phenotypes of homozygotes for those *R*(*ci*) alleles in which the right arm of chromosome 2 has been translocated to chromosome 4, broken to the right of *ci*. It should also be kept in mind that possible explanations for negative indexes may be found which may reduce the apparent shifts toward normality to unimportant phenomena due to at present unknown secondary causes.

SUMMARY

1. The degrees of vein interruption are reported for 43 unselected *R*(*ci*) alleles in heterozygotes with *ci* and +, and for 17 *R*(+) alleles selected for showing position effects in heterozygotes with *ci*. Certain *R*(*ci*) homozygotes and hemizygotes, and $R^x(ci)/R^y(ci)$ compounds have also been studied. Position effect indexes are calculated. A positive index signifies greater phenotypic deviation from normality than that of the corresponding genotype without a position allele. A negative index signifies lesser deviation from normality.

2. On the basis of several deficiencies and duplications, and of a difference in degree of position effect as related to location of a break in chromosome 4 the salivary chromosome band 102B1 or its immediate surroundings are considered as being the *ci* locus.

3. *R*(*ci*)/*ci* and R(*ci*)/+ heterozygotes from translocations of *ci* (a) to distal regions of chromosomes 2 or 3 gave striking positive position effects; (b) to proximal regions, slightly distal from the chromocenter, gave no or weak positive position effects; (c) into the chromocenter gave positive position effects of intermediate degree.

4. *R*(*ci*)/*ci* and *R*(*ci*)/+ heterozygotes involving translocations of distal or proximal plus distal parts of chromosomes 2 or 3 to chromosome 4, with a break to the right of *ci*, gave from no to intermediary position effects. In *R*(*ci*)/*ci* heterozygotes all six *R*(*ci*) alleles in which distal regions of the right arm of chromosome 2 were translocated to the *ci*-containing part of chromosome 4 gave negative position effects.

5. Genetic and cytologic tests of several *R*(*ci*) genotypes rule out triplo-4 individuals as the cause of the negative or of weak or intermediary positive position effect indexes.

6. The group of *R*(*ci*) alleles with strong or intermediary positive position effects in *R*(*ci*)/*ci* heterozygotes also gave strong or intermediary positive effects in *R*(*ci*)/+. However, within these as well as the other groups no correlation was apparent between strength of effect in *R*(*ci*)/*ci* and *R*(*ci*)/+.

7. In conformity with the results of earlier workers on *R*(+) alleles selected for showing position effects 16 out of the 17 *R*(+) alleles represented translocations of ci^+ to distal regions of chromosomes 2 or 3. The seventeenth *R*(+) allele had one of

its breaks in the left arm of chromosome 4 and a translocation of most of the right arm of chromosome 3 to chromosome 4.

8. *R(ci)* homozygotes as compared to the corresponding *R(ci)* heterozygotes were shifted toward normal. Some were even shifted toward normal beyond *ci* homozygotes (negative indexes!).

9. Some *R(ci)* hemizygotes were similar in phenotype to *ci* hemizygotes; others were completely normal.

10. $R^{z}(ci)/R^{y}(ci)$ compounds were closer to normal than one or both of the constituent *R(ci)* alleles when heterozygous with *ci*. Some compounds gave negative indexes relative to *ci/ci*.

11. In females the expressivity of *ci* and *R(ci)* in various combinations is less than in males. *R(+)/ci* genotypes tend to show a lessened difference in the degree of expressivity of the two sexes or even a reversal in direction.

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MUTATION ISOALLELES AT THE YELLOW LOCUS IN DROSOPHILA MELANOGASTER

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TIMOFÉEFF-RESSOVSKY (1932), working with *Drosophila melanogaster*, reported the existence of two distinctly different wild type alleles of the well-known sex-linked recessive gene white (w). These w^+ alleles, although phenotypically identical, could be distinguished by their characteristically different X-ray induced mutation rates. One of these alleles, w^{+A}, found in an American Drosophila stock, mutated twice as frequently as did the other, w^{+R}, present in a Russian stock. By making appropriate crosses, TIMOFÉEFF-RESSOVSKY was able to demonstrate that the difference in mutability was inherent in the alleles themselves rather than resulting from mutation modifiers of some sort. Such alleles may be termed mutation isoalleles.

No analogous demonstration of mutation isoalleles has since been reported at other loci in Drosophila. On the contrary, radiation geneticists generally assume that the mutational response to a given dose of X-rays is predictable: that a single mutation constant may be calculated that reliably expresses the probability of a mutation from one particular allelic form to another following an exposure to one roentgen unit of X-rays (see TIMOFÉEFF-RESSOVSKY and DELBRÜCK 1936). Indeed, the incidence of induced sex-linked recessive lethal mutations following a given X-ray dose has been measured by many workers, with reasonable agreement (compare TIMOFÉEFF-RESSOVSKY and ZIMMER 1939; MULLER 1950a; SPENCER and STERN 1948). Estimations of the genetic consequences of exposing human genes to ionizing radiations are predicated on the assumption that a gene responds uniformly to the mutagenic effects of unit radiation doses (see EVANS 1949; MULLER 1950b and 1954a). However, if mutation isoalleles are at all general, no such assumption is safe; and uniform mutation rates for given genes can not be expected.

At the present time it is possible to demonstrate that mutation isoalleles occur at the yellow (y^+) locus as well as at the w^+ locus.

MATERIAL AND METHODS

Several different stocks containing wild type alleles of yellow (y, locus 1–0.0) were irradiated and the number of induced y mutants was determined. Specifically, males from the following five stocks were collected and prepared for irradiation:

1. Canton-S wild type (Can-S +)
2. white-mottled-4 (w^{m4})
3. vermilion-1, forked-1 (v^1 f^1)
4. vermilion-36f, forked-36a (v^{36f} f^{36a})
5. vermilion-48a, forked-51a (v^{48a} f^{51a})

Males selected for irradiation were placed in size 000 ventilated gelatin capsules, approximately 25 per capsule. All males were less than three days old at the time of irradiation. A uniform exposure of 5000 r was given in every case, delivered at the rate of 425 r per minute, unfiltered (target distance: 35 cm; tube voltage: 250 KV; tube current: 15 ma). All males from one capsule were placed in a standard half-pint culture bottle together with about the same number of artificially virginized females which had been treated according to the technic of NOVITSKI and RUSH (1949). The females possessed the recessive sex-linked marker genes: yellow-2 (y^2), white-apricot (w^a), vermilion (v, 1–33), and forked (f, 1–56.7), as well as two inversions: scute-8 (sc^8) and delta-49 (*dl-49*). After four days in the original culture bottle, the parent flies were subcultured in a new bottle, and four or five days later the parents were discarded, thus assuring that only spermatozoa which were mature at the time of radiation were being tested. Counts of the F_1 progeny were continued for eight days after the first appearance of offspring. Between 26,000 and 66,000 F_1 females were examined in each series of exposures.

Each F_1 female exhibiting a mutant body color (or eye color) was isolated and mated with her double-inversion F_1 brothers. When such a mutant female was fertile, the nature of the genetic change could be determined from the breeding results. If mutant sons were produced, the mutation was classified as male-viable. However, a mutation could not safely be classified as male-lethal if no mutant sons appeared until outcrosses to non-inversion stocks has been carried out. The double-inversion in the parental females completely prevented crossing over between the irradiated paternal X chromosome and the unirradiated maternal X chromosome. Thus, if a male-viable mutation had occurred but was simultaneously accompanied by an independent sex-linked lethal mutation, no mutant sons would appear among the progeny of a mutant F_1 female. Likewise, no mutant sons would appear if the original mutation were truly male-lethal. However, following outcrosses to non-inversion stocks which permitted free crossing over, an accurate classification of male-lethal mutants could be made; and all suspected male-lethal yellow (and white) mutants were so tested. An exposure of 5000 r produces approximately 13.5% recessive sex-linked lethal mutations (MULLER 1950a); thus, an occasional male-viable mutant will be accompanied by an independent lethal.

In addition to fertile mutant F_1 females, some yellow mutants of good phenotype failed to produce any offspring for breeding tests. These "sterile" mutants have been taken into account in calculating the mutation rates reported in the present study. Although this procedure may not be entirely trustworthy, to ignore the non-breeding mutants entirely in calculating mutation rates would be even more unreliable, because the proportion of all mutants found that fail to breed may vary considerably from experiment to experiment. Lack of offspring may result not only from genetic sterility but also from such misfortunes as overetherization, accidental drowning of the mutant fly if the culture medium is unduly moist, or even occasionally careless handling. In addition, even though a mutant fly is fertile, sometimes only non-mutant offspring are produced. In the present study all yellow mutants that failed to produce mutant offspring, for whatever reason, have been lumped together and for convenience designated as "sterile". Unlike the situation at some other loci, only

very rarely will an apparent yellow mutant prove to have resulted from a mimicking, non-allelic mutation or from an environmental effect.

RESULTS

In table 1 are recorded all of the yellow mutants identified in F_1 female offspring following 5000 r exposures of males from each of the five stocks used. The *y* mutants are classified as: male-viable, male-lethal, and sterile. Mutation frequency at the y^+ locus is expressed in table 1 as the number of mutants expected if 100,000 F_1 offspring had been examined. It is clear that four of the five stocks showed a relatively uniform mutation frequency, giving an average of 17.4 male-viable *y* mutants and 27.1 *y* mutants of all kinds per 100,000 offspring examined. However, the fifth stock, Canton-S +, exhibited a much lower rate. Only two mutants, both male-viable, were found during an examination of 31,000 F_1 females, equivalent to an expectation of 6.5 per 100,000. This same stock was used in the extensive experiments of SPENCER and STERN (1948), who stated that in Canton-S + the mutation rate for yellow (and also for scute, miniature, garnet, and forked) was lower than that reported by workers using other stocks. However, in the present study, although few yellow mutants were found, white and Notch mutations occurred in Canton-S + at a frequency which was in no way abnormally low (see LEFEVRE *et al.* 1953). Thus, it may be suspected that certain wild type loci present in the Canton-S + stock mutate following irradiation less frequently than do the same loci present in other stocks. Such a situation parallels that reported by TIMOFÉEFF-RESSOVSKY (1932) for the w^+ locus.

TIMOFÉEFF-RESSOVSKY (1932) clearly demonstrated that the different mutation rates observed at the w^+ locus in his American and Russian stocks did not result from a mutation modifier in one of the stocks which specifically affected the mutability of the w^+ locus. In the present study no effort was made to place the various y^+ loci on a common genetic background in order to eliminate the influence of any possible mutation modifiers. However, since mutability at other loci in the Can-

TABLE 1

Yellow mutants detected in F_1 females following exposure of males from five different stocks to an X-ray dose of 5000r

♂♂ Irradiated	Yellow mutants				No. of F_1 ♀♀ examined	Expected no. ♂-viable *y* mutants per 10^5 progeny*	Expected total no. *y* mutants per 10^5 progeny
	♂-Viable	♂-Lethal	Sterile	Total			
1. $y^{36f}\ f^{36a}$	5	2	4	11	46,633	16.2	23.6
2. $y^{18a}\ f^{51a}$	6	2	3	11	45,509	17.4	24.2
3. $y^1\ f^1$	9	6	4	19	66,000	17.5	28.8
4. w^{m4}	5	4	0	9	26,164	19.1	34.4
Total (high)	25	14	11	50	184,300	17.4	27.1
5. Can-S + (low)	2	0	0	2	31,000	6.5	6.5

* A portion of the sterile *y* mutants (25/39 = 64%) has been added to the fertile male-viable mutants in each case before calculating the expectancy of male-viable mutants.

TABLE 2

Yellow mutants detected in F_1 females following exposure to various X-ray doses of males from stocks exhibiting the "high" level of mutability at the y⁺ locus

Reference	Dose in r	Yellow mutants				No. of F_1 ♀♀ examined	Expected no. ♂-viable *y* mutants per 10^5 progeny*	Expected total no. *y* mutants per 10^5 progeny, fiducial limits at 95% level of confidence		
		♂-Viable	♂-Lethal	Sterile	Total					
LÜNING 1952	960	—	—	—	33	527,497	—	4.3	6.26	8.8
HEPTNER and DEMIDOVA 1936	1000	4	0	1	5	60,530	7.8	2.7	8.3	19.3
Total	1000	—	—	—	38	571,700†	—	4.7	6.65	9.1
HEPTNER and DEMIDOVA 1936	2000	3	0	4	7	58,812	10.0	4.8	11.9	24.5
GOLDAT 1936	4000	—	—	—	42	214,403	—	14.0	19.6	26.5
GREEN, unpubl.	4000	6	4	0	10	36,651	16.4	13.1	27.3	50.2
Total	4000	—	—	—	52	251,054	—	15.5	20.71	27.2
Present study	5000	25	14	11	50	184,300	17.9	20.1	27.13	35.8
HEPTNER and DEMIDOVA 1936	6000	9	0	5	14	46,800	26.9	16.3	30.0	50.3

* A portion of the sterile *y* mutants (47/65 = 72%) has been added to the fertile male-viable mutants in each case before calculating the expectancy of male-viable mutants.

† Data of LÜNING have been adjusted to 1000 r on the basis of the curve shown in figure 1.

TABLE 3

Yellow mutants detected in F_1 females following exposure to various X-ray doses of males from stocks exhibiting the "low" level of mutability at the y⁺ locus

Reference	Dose in r	Yellow mutants				No. of F_1 ♀♀ examined	Expected no. ♂-viable *y* mutants per 10^5 progeny*	Expected total no. *y* mutants per 10^5 progeny, fiducial limits at 95% level of confidence		
		♂-Viable	♂-Lethal	Sterile	Total					
DEMEREC 1934	2500	1	1	0	2	37,346	2.7	0.6	5.4	19.3
GLEMBOZKY 1936	4000	8	0	5	13	176,421	7.2	3.9	7.4	12.6
HEPTNER and DEMIDOVA 1936	4000	6	0	5	11	106,674	10.0	5.2	10.3	18.5
Total	4000	14	0	10	24	283,095	8.3	5.4	8.5	12.6
Can-S + (Present study)	5000	2	0	0	2	31,000	6.5	0.8	6.5	23.3

* A portion of the sterile *y* mutants (17/18 = 94%) has been added to the fertile male-viable mutants in each case before calculating the expectancy of male-viable mutants.

ton-S + stock was not affected at the same time that so few mutations at the y^+ locus occurred, obviously Canton-S + contains no mutation modifiers affecting overall mutability. Although the possibility remains that Canton-S + has a mutation modifier that specifically reduces X-ray induced mutability at the y^+ locus, none of the mutation modifiers so far described in Drosophila, even though significantly affecting spontaneous mutability, has been shown to modify X-ray induced mutation rates. Thus, it seems plausible to assume that the different mutability levels exhibited at the y^+ locus reflect differences inherent in the locus itself—not differences impressed on the locus by independent mutation modifiers.

An effort has been made to verify the existence of two distinctly different rates of X-ray induced mutability at the y^+ locus by searching the literature for reports of the frequency of yellow mutations produced following exposure to various X-ray doses. Several such reports exist supporting the thesis that two levels of mutability occur at the y^+ locus, and the results combined with those from the present study are listed in tables 2 and 3. The mutation rates clearly fall into two non-overlapping groups, high and low. The actual mutation frequencies, expressed as the expectation per 100,000 gametes tested, may be more readily appreciated by reference to figure 1.

In figure 1 the average total mutation frequency at each X-ray dose is plotted against the dose. Data on which the "high" curve is based are found in table 2,

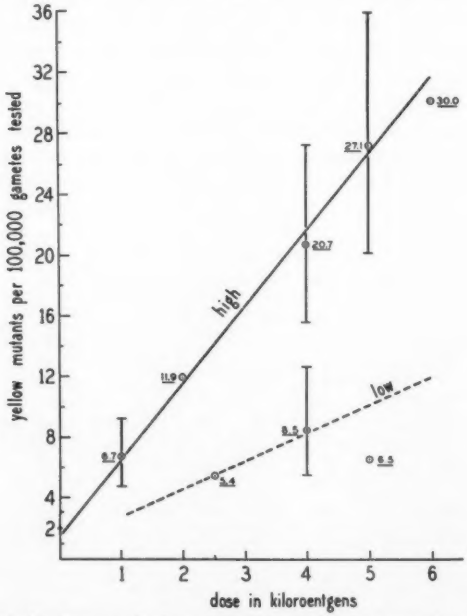

FIGURE 1. Relationship between the frequency of induced yellow mutations and the X-ray dose applied, showing two non-overlapping levels of mutability. (Data for the "high" curve are from table 2, for the "low" curve from table 3.)

while the "low" curve is plotted from the data in table 3. The fiducial limits indicated in figure 1 represent the 95% confidence level and were calculated with the aid of STEVENS' (1942) tables. The points for which fiducial limits are not shown are based on such small numbers of mutants (2 to 14) that the limits are extremely broad; thus, very little significance can be ascribed to those points. Nonetheless, it is of interest to note that, despite their unreliability, they fall close to the curves, which were plotted from the more reliable points alone.

The "high" curve was fitted by the method of least squares to the points at 1000 r, 4000 r, and 5000 r. The y-intercept indicates that the spontaneous mutation rate at the y^+ locus is about 1 per 75,000. If this value seems somewhat high, it might be noted that spontaneous *reverse* mutation at the forked locus can be equally high, if not higher (RHOADES 1931; LINDSLEY personal communication; GREEN personal communication). MULLER (1950b) considered that the average spontaneous mutation rate for Drosophila loci is about 1 per 100,000. Actually, the present data are not sufficiently reliable to place undue significance in the exact value of the y-intercept and hence in the spontaneous mutation rate at the y^+ locus. Indeed, there is no assurance that the spontaneous mutation rate was the same in all experiments.

The true slope of the "low" curve can not be determined with any great accuracy, and the plot shown in figure 1 has simply been fitted to the 4000 r point. Only that point is reasonably reliable, but it is significant that the mutation rate indicated by that point does not overlap the high mutability rate at 4000 r. The points at 2500 r and 5000 r by themselves would not prove that two mutability levels exist. However, they do support the 4000 r data; and with all information taken into consideration, mutational isoallelism at the y^+ locus may safely be inferred. The high rate, to be sure, appears to represent the more typical mutation level of the y^+ locus, but the occasional low mutation rates that have been reported do not represent random variations in a uniform mutation rate.

From figure 1 it can be estimated that the high mutability level is appreciably more than twice the low rate. However, if only male-viable mutability is considered (as was the case in the studies on the white locus by TIMOFÉEFF-RESSOVSKY) rather than total mutability at the y^+ locus, then the difference in the two levels is not so great. Reference to tables 2 and 3 shows that a considerably greater fraction of all fertile yellow mutants in the high group were male-lethal (18 out of 65) than in the low group (1 out of 18). Actually, male-viable mutability at the high level is only slightly more than twice that at the low. These relations can be determined by calculating the appropriate mutation constants. Considering the data at the high level, the mutation constant for male-viable mutability is about 4×10^{-8}; that for total mutability, 5.5×10^{-8}. At the low level, similar values are 1.9×10^{-8} and 2×10^{-8}, respectively.

DISCUSSION

Linearity of mutation data at invidiual loci

Anyone who wishes to learn the precise rate at which a particular gene mutates under the influence of given X-ray doses will be distressed by the number of different

answers he can find in the literature, limited by and large only by the number of different references he examines. Finding reports by two different investigators that agree fairly well regarding a particular mutation rate is rather more the exception than the rule. This lack of uniformity in mutation data stems from several obvious causes.

First, the detection of visible mutations is much more subjective than is the detection of lethal mutations, where, by contrast, published data on mutation rates are in much better agreement than are data on mutation at individual loci.

Second, all investigators do not use the same experimental technic nor follow the same procedure in computing mutation rates at individual loci. Some base the rates on the frequency of induced fertile male-viable mutations (for example, VALENCIA and MULLER 1949). Some report mutations with no breakdown into viable, lethal, and sterile categories (for example, BONNIER and LÜNING 1949). Some, detecting mutations in F_1 males (rather than in females) as the result of matings with attached-X females, necessarily report only male-viable mutations not simultaneously accompanied by an independent sex-linked lethal mutation (for example, TIMOFÉEFF-RESSOVSKY 1932). Others attempt to identify all detectable mutations of a particular kind in order to establish the mutation rate, as was done in the present experiments. Thus, adequate comparisons of mutation rates at individual loci can be made only when the experiments were carried out and the data were treated in the same manner.

Finally, mutation rates at individual loci are inevitably low. Therefore, even though large numbers of progeny are inspected, the total number of mutants found is certain to be relatively small. No one who undertakes a mutation experiment, spending months examining perhaps a hundred thousand individuals in order to establish a single point on a dose-rate curve, can escape a feeling of futility when the fiducial limits of that "point" are calculated. Chance alone can be expected to produce a marked effect on the calculated mutation rates, thus giving rise to non-uniform mutation data.

Despite the variability of the mutation data, the proposition that individual gene loci, like the constellations of lethal loci, exhibit linear X-ray induced mutation rates in Drosophila has not been seriously questioned. Mutation constants for various mutation steps have been calculated by TIMOFÉEFF-RESSOVSKY and DELBRÜCK (1936), and as a rough guide to the mutational expectation of the particular steps involved, these constants still serve their purpose. However, BONNIER and LÜNING (1949) reported data on mutation at the white and forked (*f*) loci which are difficult to interpret. Their dose-rate curves did not pass through the origin. Indeed, they passed through the y-intercept significantly above the level of the spontaneous mutation rates exhibited by their controls. No simple mutation constant can express the mutational expectation under such circumstances, and their work has cast doubt on the view that induced mutability at individual loci in Drosophila is directly proportional to the X-ray dose applied. MULLER (1954b), commenting on the results of BONNIER and LÜNING, suggested that their control data were invalid; that in reality the spontaneous mutation rate was higher than they thought. Generally control cultures are much more crowded than irradiated cultures. Mutants, then, may well be at a greater selective disadvantage in an overcrowded control culture than in an

experimental culture, thus giving rise to a spuriously low spontaneous mutation rate. However, a spontaneous mutation rate of approximately 5 per 100,000 would be required to fit the curves of BONNIER and LÜNING, and a value that high is assuredly not to be expected at either the w^+ or the f^+ locus. Moreover, such a crowding effect should be greater in the low-dose cultures than in the high-dose ones, so that the dose curves would tend to be depressed at low dosages. Since the contrary was the case, it seems unlikely that the results of BONNIER and LÜNING can be explained by recourse to a population effect on the survival of mutants under different conditions.

A second explanation for the unusual data reported by BONNIER and LÜNING might be found in a consideration of mutational isoallelism. If the stocks they irradiated actually possessed more than one w^+ and f^+ mutation isoallele, chance might have produced mutation curves such as they found. In fact their data for forked mutations are particularly suggestive of just such a situation. At two of the four dose levels they tested, the *f* mutation rate is well below the curve, and at the other two points, well above. If the four values they reported do not represent random variations in a uniform mutation rate, but rather two distinctly different rates, then their data no longer conflict with a direct proportionality of X-ray induced mutation to dose. In this connection it should be noted that the data of HEPTNER and DEMIDOVA (1936) cited in tables 2 and 3 exhibit a similar lack of uniformity. At 1000 r, 2000 r, and 6000 r yellow mutants were clearly produced at the "high" rate; but at 4000 r, even though twice as many progeny were examined as at the other dose levels, the mutation rate was characteristic of the low group. At the same time, however, at 4000 r they found no comparable scarcity of white, forked, or Notch mutants.

It is natural to assume that both BONNIER and LÜNING (1949) and HEPTNER and DEMIDOVA (1936) exposed only a single stock throughout their radiation studies, although that is not necessarily the case. Thus, it may seem unreasonable to invoke mutation isoalleles in order to explain their unexpected results. However, a given stock need not be homozygous for a particular mutation isoallele. Should two (or more) different mutation isoalleles occur at a given locus in a stock, some intermediate level of mutability would be exhibited, depending on the relative frequencies of the isoalleles. Over the course of time, the relative frequencies of mutation isoalleles in a particular stock could easily change through genetic drift.

If neither this explanation of BONNIER and LÜNING's data nor that of MULLER (1954b) is acceptable, there remains the view that mutation data on individual loci are inherently so unreliable that uniform results cannot be expected, even though exceedingly large scale experiments are carried out. Fortunately, the present study provides a clear measure of the uniformity that can be expected in essentially duplicate mutation experiments. Where statistically significant differences occur in the mutation rates at individual loci, the most plausible explanation is the unsuspected occurrence of two or more different mutation isoalleles in the stock or stocks under investigation. The subjective nature of work on mutation rates at individual loci, the effect of chance on low mutation rates, and the effect of culture conditions on the survival of mutants will produce variability in mutation data; but they will not all operate in the same direction, and they will not give rise to two distinct, non-over-

lapping levels of mutability at a given locus. The serious inconsistencies in the published data on mutation rates at individual loci may best be rationalized by applying the concept of mutational isoallelism.

The studies of GILES (1951, 1953) on mutability at the inositolless locus in Neurospora indicate that mutation isoalleles occur there, as judged by the significantly different rates of reversion exhibited by different inositolless alleles. It may be suspected that mutation isoalleles are widespread and must be taken into consideration in the analysis of any mutation study. Whatever the true situation may be, little doubt can remain that the mutational response of a particular allele is linear with the radiation dose.

The nature of mutation isoalleles

Accepting the existence of mutation isoalleles, it remains to be explained how two forms of the same gene can arise. What is the actual difference between mutation isoalleles that accounts for their different mutabilities? Some years ago the explanation would have been simple. Under the target theory (see TIMOFÉEFF-RESSOVSKY, ZIMMER, and DELBRÜCK 1935) the mutability of a gene is a measure of its size. If it were assumed that a gene could exist in a compact as well as in an extended state, then the occurrence of mutation isoalleles could readily be understood. However, the target theory can no longer be upheld, and the indirect action theory has taken its place (for a complete discussion, see MULLER 1954b). Therefore, an explanation for mutation isoalleles must be sought in different terms than gene size.

Under the indirect action theory, ionizing radiation affects genes indirectly following their reaction with active radicals, HO_2, or H_2O_2 formed in water surrounding the gene. Since unit X-ray doses produce similar concentrations of such reactants (assuming normal temperature and oxygen tension conditions), then the mutability of a given gene should be related to its reactivity. If the reactivity of a gene can somehow be altered without change in its specificity, then mutation isoalleles could be explained. However, since the reactants produced in water by ionizing radiation are mainly strong oxidizing agents, it is difficult to visualize the kind of gene change that would be required in order to alter the reactivity with such agents and yet which would preserve unaltered the essential function of the gene. Still, if such a change can occur, then an interesting alternative exists. Should an immediate gene neighbor of the locus under consideration suffer a change in reactivity, there might result a somewhat altered concentration of the radiation-produced reactants near the locus. Thus, mutation isoallelism would reflect the consequence of a mutation in a neighboring gene, not in the locus being studied, giving rise to a novel position effect on mutability.

A more plausible interpretation of mutation isoalleles might possibly be found in a consideration of the pseudoallelic nature of many loci in Drosophila (LEWIS 1945, 1950, 1951; GREEN and GREEN 1949; GREEN 1954). Pseudoallelic genes apparently arise following duplication of an originally unitary locus. Thus, instead of the usual symbolization, m^+ for example, pseudoallelic loci must be designated as m^+m^+. Either of two mutational changes can give rise to a mutant: mm^+ or m^+m. If at the yellow locus (where pseudoallelism has not yet been demonstrated) and at the white

locus (where it has: LEWIS 1952; MACKENDRICK and PONTECORVO 1952) all wild type alleles are *not* duplicated; if in fact some stocks contain unitary y^+ or w^+ loci and others have duplicated pseudoallelic y^+y^+ or w^+w^+ loci, then mutation isoallelism could readily be explained. A doubled opportunity for a given mutation would exist at a duplicated pseudoallelic locus as compared with that at a unitary locus. The fact that pseudoallelism has been demonstrated at a particular locus is in itself no proof that every wild type allele of that locus is pseudoallelic. The duplicated condition may never have become completely established in the species; or even though once established, the duplication may have been lost in some strains, thus restoring the unitary condition. If this is the case, male-viable mutation should be twice as frequent in a stock containing a pseudoallelic locus as in a stock with a unitary locus. However, it is difficult to predict the relative frequencies of male-lethal mutation in the two cases. Deficiency of the yellow locus (MULLER 1935) and of the white locus (PANSHIN 1938) is not necessarily lethal. Probably male-lethal *y* or *w* mutations actually involve a loss of more than the single gene. If so, the frequency of male-lethal deficiencies produced following irradiation of a pseudoallelic locus might be the same as the frequency found after exposure of a unitary locus; but it might equally well be quite different, depending on a variety of uncertain conditions. At the yellow locus the evidence from the present analysis indicates that male-lethal *y* mutants are considerably more frequent at the high level of mutability (= pseudoallelic?) than at the low. Of course, any interpretation of mutation isoalleles on the basis of a pseudoallelic duplication versus a unitary locus is merely a sophisticated way of saying that some genes may be bigger than others.

Some loci such as white, lozenge (*lz*), vermilion (*v*), and forked, among others, typically exhibit relatively greater induced mutability than do other loci (such as raspberry or achaete). The fact that *w*, *lz*, *v*, and *f* are known to be pseudoallelic (LEWIS 1952; GREEN and GREEN 1949; GREEN 1954) may well explain why their mutation rates should be high as compared with other loci which may not be pseudoallelic (see discussion of ALEXANDER 1954). Even if all loci were pseudoallelic, differences in mutability between entirely different loci could be related to a greater degree of pseudoallelic duplication in some loci than in others. It should be noted that even the "high" overall mutation rate at the y^+ locus is less than half that characteristic of the w^+ locus, which itself is only half that exhibited by certain w^+ isoalleles (TIMOFÉEFF-RESSOVSKY 1932; and unpublished data).

Use of the concept of "degrees" of pseudoallelism to explain mutation isoalleles may be open to question. Any mutant arising at a unitary locus would be incapable of recombination with alleles arising independently in the same chromosome. Moreover, it is doubtful whether one-locus mutants could cross over with alleles occurring at a duplicated locus. At the loci best studied for pseudoallelism (Star-asteroid, LEWIS 1945; lozenge, GREEN and GREEN 1949; white, LEWIS 1952) the evidence indicates that each independent allele thoroughly tested will cross over with some other allele. Thus the implication is strong that each mutant arose at an already pseudoallelic locus. However, if a one-locus allele *could* cross over with a two-locus allele, then the one-locus allele should show recombination with both "left" and "right" two-locus alleles and thus pass as a "middle" allele. In addition, should

unitary and duplicated alleles (whether mutant or non-mutant) occur in the same stock, crossing over could convert one into the other. At the present time, however, no evidence provides direct support for the view that different degrees of pseudoallelism can exist.

If the presence of intercalary heterochromatin near a locus affects its mutability as claimed by PROKOFYEVA-BELGOVSKAYA (1941; see also discussion of HANNAH 1951), then a third interpretation of mutation isoalleles is possible. PROKOFYEVA-BELGOVSKAYA pointed out that intercalary heterochromatin was found near several of the more mutable loci in Drosophila, namely yellow, white, cut, lozenge, forked, and bobbed. SIDOROV (1936), BELGOVSKY and MULLER (1938), MULLER (1940), PANSHIN *et al.* (1946), and LEFEVRE *et al.* (1953) have demonstrated that the mutability of genes moved by inversion from a euchromatic neighborhood to a heterochromatic one is markedly increased. However, most of the increase is evidenced as more male-lethal mutations. If a locus possesses some characteristic level of mutability when no heterochromatin whatever is present, then the insertion of intercalary heterochromatin nearby would presumably increase the mutability to a degree proportionate to the amount of heterochromatin present. Since heterochromatin is genetically "inert", the amount of intercalary heterochromatin present next to a locus may well change from time to time through deficiency or insertion. Thus, in different stocks the quantity of intercalary heterochromatin adjacent to a particular locus may be quite different. When heterochromatin is present, adjoining euchromatic loci are subject to an increased likelihood of loss as compared with the situation where little or no heterochromatin is nearby (LEFEVRE *et al.* 1953). However, since loss of either the y^+ or the w^+ locus is not necessarily lethal, an increase of both male-viable and male-lethal mutability would be expected when intercalary heterochromatin is present. At the y^+ locus viable and lethal mutability are greater in the high group (having intercalary heterochromatin?) than in the low.

In connection with the discussion of gene mutability and reactivity, the influence of heterochromatin on mutability could stem from different reactivities of heterochromatin and euchromatin with radiation-produced mutagens. Many levels of mutability at a given locus might be found in different stocks if the quantity of adjacent intercalary heterochromatin can vary to any degree. However, if heterochromatin occurs only in blocks (MULLER and GERSHENSON 1935), then relatively few different mutability levels would be expected at a given locus.

Of the three possible explanations for mutation isoalleles offered above, the last appears to agree best with the facts regarding mutation at the yellow locus. However, no definite choice can be made. It should be noted that at the white locus, although the existence of two mutability levels can be verified, male-lethal mutability appears to be constant and only the male-viable mutability differs (unpublished data). Whatever the true explanation for mutation isoalleles may be, their existence can scarcely be questioned. Under any view there is no reason to believe the yellow and white loci are unusual; thus mutation isoalleles may be expected to occur generally. If so, the genetic consequences of exposing organisms (including humans) to radiation become much more difficult to predict.

SUMMARY

Drosophila melanogaster males of five different stocks containing wild type alleles of yellow were exposed to 5000 r X-ray doses, and F_1 female offspring were carefully examined for the presence of *y* mutations. The frequency of *y* mutants was quite similar in four of the stocks, but was considerably lower in the fifth. Reports from the literature on the frequency of mutation at the y^+ locus following exposure to various X-ray doses support the suggestion that two non-overlapping levels of mutability occur at the y^+ locus. Thus, as at the w^+ locus, mutation isoalleles exist at the y^+ locus.

The existence of mutation isoalleles may be explained by assuming that: 1) the reactivity of genes with radiation-produced agents such as H_2O_2, HO_2, or active radicals can vary without affecting gene specificity, 2) that the degree of pseudoallelic duplication at loci such as y^+ or w^+ is not uniform throughout the species, or 3) that intercalary heterochromatin adjacent to loci such as y^+ or w^+ influences their mutability and that the amount of intercalary heterochromatin is variable. In any event a general occurrence of mutation isoalleles may be suspected. If so, predictions regarding the mutational effect of given radiation doses are subject to additional uncertainties.

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AN UNSTABLE NUCLEAR CONDITION IN TETRAHYMENA PYRIFORMIS[1]

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CONJUGATION among the cells of a single clone has been observed in many Ciliates, including those in which mating types have been demonstrated. In forms for which mating types have not been established, two interpretations of intra-clonal conjugation are possible: either the cells that unite in conjugation are physiologically identical, or they are physiologically distinct (of diverse mating types) but the physiological distinctions are transitory. In forms with mating types, intra-clonal conjugation has usually been shown to involve the union of cells of diverse physiological types (KIMBALL 1939; JENNINGS 1941) which may be perpetuated with some degree of reliability in vegetative growth. Since mating types are involved in the "selfing" of some Ciliates, it has been proposed (KIMBALL 1943) that selfing in other Ciliates has a similar basis and that all, or nearly all, Ciliates possess mating types. This is, however, only the simplest interpretation and must be accepted with reservation.

Whatever may be the explanation for selfing in the majority of Ciliates, the presence of diverse cell types in clonal cultures of Paramecium (KIMBALL 1939) and Tetrahymena (NANNEY and CAUGHEY 1953) provides an opportunity for the study of cellular differentiation and cellular heredity. More specifically, selfing cultures present a special problem in an understanding of mating type determination and inheritance in these forms. Recently (NANNEY 1953a) a formal explanation for the selfers in *P. aurelia* was presented. The present communication, based on studies of selfers in Tetrahymena, suggests that the explanation proposed for *P. aurelia* is not applicable to Tetrahymena and that it may not be applicable to Paramecium.

MATERIALS AND METHODS

Strains of the taxonomic species *Tetrahymena pyriformis* may be divided into three general groups: 1) those in which conjugation has never been observed, 2) those in which conjugation usually occurs only when clones are mixed in certain combinations and 3) those in which conjugation occurs regularly in clonal cultures. The present report is based on a series of strains belonging to the second group and isolated from a source near Woods Hole, Massachusetts, by ELLIOTT and GRUCHY (1952). From two of these original strains (WH-6 and WH-14) 7 different pure mating types have been derived (NANNEY and CAUGHEY 1953). These types were designated by Roman numerals I–VII. ELLIOTT and GRUCHY designated the first types as I and II and these are homologous with types I and II in the system of 7 types, but ELLIOTT and

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HAYES (1953) have subsequently reported a third mating type, mating type III, isolated from the same locality. This type III has been found to be homologous with type VI in the NANNEY-CAUGHEY system. In order to avoid further confusion in the literature, the designations III and VI in the NANNEY-CAUGHEY system have been exchanged. Therefore, the mating type designations in this and subsequent papers should be equivalent to those used by ELLIOTT and his co-workers.

Although these forms will grow on a defined medium (ELLIOTT and HAYES 1953), most of the work reported here was carried out on cultures grown in Cerophyl infusions inoculated with *Aerobacter aerogenes*. In a few of the experiments Cerophyl was replaced with baked lettuce or *Aerobacter aerogenes* was replaced with *Bacillus cadaveris*. The general culture methods follow closely those described by SONNEBORN (1950) for *Paramecium aurelia*.

The cytological events at conjugation in these strains (ELLIOTT and HAYES 1953; NANNEY and CAUGHEY 1953) resemble closely those described for *Leucophrys patula* (MAUPAS 1889) and for the AA strains of *Tetrahymena* (NANNEY 1953b). Cytological studies of clones under various conditions have failed to provide evidence for autogamy. An immature period occurs immediately following conjugation and is terminated by about 80 fissions. The procedure used to obtain mature exconjugant clones is to allow the exconjugants to pass through a series of isolation cultures, single cells being transferred to fresh medium in depression slides every day or two. After 24 hours at 26°C the cells have usually undergone about 10 fissions and are still actively dividing; after 48 hours they have undergone about 12 fissions and are moderately starved. The cultures from which isolations have been made ("left-over" cultures) are retained for several days and are observed periodically for selfing. Under these conditions the clones become mature after about 8 transfers and are then tubed.

Subsequently samples of each tube culture are mixed with samples of each of the seven standard mating types in order to determine the mating type of the clone. Each mixture consists of about 0.25 ml of the well-fed unknown culture and an equal quantity of a well-fed standard type. The controls consist of the unmixed unknown culture, the unmixed standards and mixtures of the standards in all 21 combinations of 2. Since mating does not occur immediately, the tests are examined for conjugants at several times on the following day. Pairs usually form over a period of several hours and may remain together as long as 18 hours; hence, it appears unlikely that pairs occurring in a mixture will fail to be observed. An unknown culture is classified as of a particular type if it fails to mate with the standard of that type, but mates with all the other mating types. A culture is classified as a selfer if pairs are formed in the unmixed control; such cultures also show pairs in all the mixtures with the standards. Many selfers are detected before mating type tests are set by observing the left-over cultures in the isolation series. A small fraction of the cultures may remain undetermined on the first test, i.e., they may fail to mate with two or more of the standards. When such cultures are fed in the test depressions with inoculated culture medium, their mating types may usually be assigned on the following day.

OBSERVATIONS

The nature of selfing clones

Nearly all the crosses thus far made have yielded some selfing clones. The first question raised concerns the reason for the selfing: are the cells that conjugate in clonal cultures of the same or of different mating types? Evidence has been presented previously (NANNEY and CAUGHEY 1953) to show that when cells of diverse mating types are mixed, conjugation occurs only between cells of unlike mating type. In light of this evidence the simplest explanation for conjugation in clonal cultures is that these clones contain cells of two or more mating types. This explanation is supported by the fact that stable cultures of several different mating types have been derived from some of the selfing clones. Single cells isolated from selfing cultures yield some sub-cultures which again self, and some sub-cultures which are pure for some mating type and remain so. (Still other isolations yield immature clones; such isolations are obtained only from cultures in which conjugation has been permitted and are presumably due to the existence of exconjugant cells in the cultures. Such isolations are excluded in the following discussion.) The 5 pure sub-cultures from one selfing clone included 4 different mating types—II, IV, V and VI. The 9 pure sub-cultures from another clone also included 4 types—III, IV, VI and VII. Sub-cultures from other selfing clones yielded 2 or 3 different mating types in various combinations and with various relative frequencies. In these cases it is clear that a diversity of types occurs within the cultures and that this diversity is sufficient to account for the observed conjugation.

These observations do not, however, rule out the possibility that some of the conjugation observed in selfing clones is due to the union of cells of the same mating type. This possibility would not require serious consideration were it not for the fact that it has not been possible to derive diverse pure types from some of the selfers. In several instances where 15 to 30 pure sub-cultures were derived from a selfer, all were of the same mating type. The selfer most intensively studied produced over 200 pure sub-cultures, all of mating type VI. It is possible that cells conjugating in these clones have the same mating type, but this conclusion is not required. The failure to establish pure cultures of more than one mating type from a selfer may be due to the rarity or the instability of one or more of the types. If a particular type were rare, it would be selectively involved in conjugation so that among the cells which were not conjugating at the time isolations were made, it would be virtually absent. If a particular type were unstable, an isolated cell manifesting that type might again give rise to a selfing culture or even a pure culture of a different type. Since diverse mating types have been shown to occur in some selfing cultures and since no convincing evidence is available for conjugation involving cells of the same mating type, it will be assumed tentatively in the following discussion that two or more mating types occur in all selfing cultures.

The origin of mating type diversities in selfing clones

The second question raised in regard to the selfers concerns the manner in which the diversities arise. Mating types are known to change at autogamy in *P. aurelia*

(KIMBALL 1937; SONNEBORN 1947). Since the mode of mating type determination in Tetrahymena resembles so closely that in *P. aurelia*, it would be expected that autogamy, if it occurred in *T. pyriformis*, would result in the formation of diverse mating types. Therefore, attempts have been made to detect autogamy in the WH strains and particularly in the selfing clones. Several clones in which selfing had been observed in left-over cultures were chosen for study. Single cell isolations from well-fed selfers usually give rise to cultures which self extensively when allowed to starve. If autogamy is to account for this selfing, it must occur between the time a single cell is isolated and the time the culture starves some 12 fissions later. Large numbers of cells were removed from such cultures both while they were growing rapidly and after they had begun to starve. No evidence was found for reorganization in unpaired cells, yet extensive selfing, involving more than half the cells, occurred in all the cultures studied. Diverse pure types were subsequently obtained from some of these same cultures. While it remains possible that autogamy occurs rarely in these strains, it must be concluded that autogamy is not a major factor in rendering cells in selfing cultures capable of conjugating.

Finally it is possible that conjugation (or some other process of nuclear reorganization occurring at the time of conjugation) is the source, rather than the result of mating type diversities. This is considered unlikely in view of the fact that conjugation normally results in cells which are immature for long periods of time; the diversities now known to be produced at conjugation cannot account for the diverse mature types derived directly from selfing clones. It was considered necessary, however, to obtain diverse pure cultures from selfers in which conjugation had not yet been permitted. This was accomplished as follows. Clones were maintained in daily transfers until selfing was observed in left-over cultures. When selfing was observed, single cells were isolated from the next culture in the isolation series, a culture in which the cells were well-fed and in which conjugation had not yet occurred. Under such circumstances relatively few cells give rise to pure cultures, but cultures of diverse pure type were obtained from some of the selfers. This shows clearly that neither conjugation nor some other process of reorganization associated with conjugation is solely responsible for the mating type diversities observed in selfing clones. The diversities appear during cytologically normal vegetative growth.

The stabilization of selfing clones

The experiments mentioned in the previous paragraph also demonstrated another fact—that cells isolated from selfers under different conditions yield different frequencies of pure cultures. 208 cells isolated from 7 different well-fed selfers produced only 14 (7%) pure cultures. 204 cultures derived from these same clones when starved included 58 (28%) pure cultures. Every selfer examined produced a higher frequency of pure cultures from starved cells than from well-fed cells.

At least two explanations for this observation seem possible. Either starvation (or some condition associated with starvation) is responsible for stabilizing the mating types of cells in selfing clones, or those cells more likely to give rise to selfing cultures are also more likely to participate in conjugation and are, hence, selected against in making isolations from cultures in which conjugation is occurring or has occurred,

i.e., starved cultures. This latter explanation is rendered improbable by the fact that cells can be stabilized by starvation under conditions which permit no conjugation and, hence, no obvious selection. 312 cells from a total of 15 well-fed selfers were isolated into culture medium and allowed to grow; only 21 (7%) of the derived cultures were pure for a mating type. 416 cells from these same cultures were individually isolated at the same time in separate drops of distilled water and allowed to starve; these cells, after being isolated into culture medium, produced 119 (29%) pure cultures. Such observations strongly indicate that starvation may stabilize potential selfers and that the starvation effect is not due to crowded culture conditions or accumulated metabolites.

Since starvation was shown to have a marked effect on selfers after the onset of selfing, it was considered of interest to determine whether a starvation effect could also be demonstrated at a time before overt selfing had occurred. This possibility was first examined by differential treatment of the progeny of unselected crosses. In one series of 15 crosses, representing 11 different parental mating type combinations, the exconjugant clones were transferred daily for ten days as single cells to new media and were then tubed and tested for mating type. During the growth period the clones were not allowed to starve, except in the left-over cultures. The left-over cultures were examined for conjugants every 24 hours during the interval in which they were starving. Of the 360 clones studied, 69 (19%) were observed selfing after they became mature.

The progeny of another series of 28 crosses, representing 14 combinations of parental mating types, were treated in precisely the same fashion as the previous series with the exception that single cells were transferred on alternate days, after the isolation cultures had begun to starve. Only 16 (2%) of the 758 clones were selfers at the time of maturity. These experiments strongly indicated that starvation during growth had an effect on the frequency of selfing detected after maturity. Certain criticisms can, however, be raised against such data, and certain questions remain unanswered. The chief criticisms are concerned with the fact that the crosses used in the two series were not precisely comparable and that the experimental clones were studied at different times over a period of several months. Moreover, the numbers of progeny in the individual crosses were so small as to make difficult any test of homogeneity within the two series. An experiment was designed, therefore, specifically to answer the question of whether starvation prior to selfing had an influence on the incidence of selfing.

For this experiment a cross was made between sister caryonides of a highly inbred strain; the exconjugants and the first fission products were separated to provide 4 caryonides from each original pair. After the caryonides had undergone about 10 fissions two cells were isolated from each caryonidal culture to give rise to two subcultures designated as A and B. The A sub-cultures were subsequently transferred daily to new depressions until they had undergone a total of 60 fissions. The B subcultures were transferred at 3-day intervals until they had also undergone a total of 60 fissions. After 60 fissions both the A and B sub-cultures were again split into two further sub-cultures, A-1 and A-2, B-1 and B-2. The A-1 and B-1 cultures were maintained thereafter in daily isolations until they had undergone a total of 160 fissions

from conjugation. The A-2 and B-2 cultures were transferred at 3-day intervals until they also had undergone a total of 160 fissions. All 4 series were examined at 12 hour intervals as the left-over cultures starved in order to detect the occurrence of selfing. The A-1 series, which was not allowed to starve at any time during the 160 fissions, produced 77 (56%) selfers among 138 surviving clones. The A-2 series, which was permitted to starve only after the first 60 fissions, produced 21 (16%) selfers among 134 clones. The B-1 series, starved only before the 60th fission, produced only 5 (4%) selfers among 136 clones, and the B-2 series, starved at every transfer, produced 9 (7%) selfers among 131 clones. These results leave little doubt that starvation prior to the onset of selfing decreases the observed frequency of selfing.

The relationship between maturity and instability

This experiment also yields information regarding the time when starvation can be effective in relation to the end of the period of immaturity. The cross used was one which could not yield mating type I; in order to detect the onset of maturity, the left-over cultures in the A-1 and B-1 series were mixed with well-fed mating type I cultures. After 40 fissions in the A series practically none of the clones was reactive; after 50 fissions a few (16%) were beginning to be reactive; after 60 fissions 43% were reactive to some extent and after 70 fissions 83% were reactive; after 80 fissions all the clones were completely reactive. In terms of fissions, maturity arrived somewhat earlier in the B series; all the B clones were completely reactive by 60 fissions, the first time they were tested. The rationale for using the 60th fission as a point of bifurcation in the experiment was to determine whether starvation was effective in preventing selfing if applied either before or after the onset of maturity. Since maturity does not arrive abruptly, when considering either the total series or individual clones, 60 fissions is only an approximation of the transition from immaturity to maturity. Nevertheless, the data strongly suggest that starvation either before or after the beginning of maturity is effective in stabilizing potential selfers.

This experiment also shows one additional characteristic of the selfers, the relationship between the onset of maturity and the onset of selfing. Again referring to the A-1 series, selfing was never observed as soon as the culture became mature. Some clones (about 10% of the detected selfers) conjugated in the isolation culture immediately following the one for which maturity was first demonstrated. These clones may have included diverse mating types when they became mature and the failure to detect pairs earlier may have been due to their relative immaturity in the previous isolation culture. This conclusion appears much less likely in the case of clones which did not self for many fissions after they became mature. Three clones selfed for the first time 90 fissions after they became mature and the average number of fissions between the onset of maturity and the detection of selfing was about 40 fissions. It is conceivable that some of this apparent lag between maturity and selfing was due to faulty observation, but since the left-over cultures were examined carefully at 12 hour intervals it seems improbable that any large amount of the selfing was missed.

DISCUSSION

The physical basis for selfing in the Ciliates

A previous attempt (NANNEY 1953a) to provide a formal explanation for the occurrence and behavior of selfers in *P. aurelia* was based on the following considerations. 1) Mating types in this species are controlled by the macronuclei. The studies of SONNEBORN (1947, 1951) have provided abundant evidence for this conclusion, the chief evidence being that in certain stocks new mating types arise only when new macronuclei are formed and the separation of diverse mating types occurs only when the new macronuclei are segregated, i.e., at the first fission following nuclear reorganization when caryonides are separated. 2) The exceptions (rare in some stocks and varieties and common in other stocks and varieties) were considered exceptions only in a qualified sense. Selfing, like mating types, was thought to be a caryonidal characteristic and, hence, related to some macronuclear peculiarity. Critical evidence is not available to show that selfing in *P. aurelia* is caryonidal, but the fact that mating types are controlled by the macronuclei suggests that the aberrant mating type control in selfing clones is related to some macronuclear peculiarity. 3) A macronucleus undoubtedly contains many sets of nuclear genes (SONNEBORN 1947). The manner in which these are organized in the macronucleus is not known, but it is possible that they form sub-structures with some degree of integrity. 4) If the unit of mating type determination were the sub-nuclei rather than the macronucleus as a whole, two classes of macronuclei would be conceivable: those that consist of a single type of sub-nucleus, determine a single mating type and reproduce true to type and those that contain more than one type of sub-nucleus, which have the potentialities for more than one mating type and which can produce, through the segregation of sub-nuclear units at fission, cells of diverse mating types. This interpretation held that the "instability" of selfing clones was due to structural inhomogeneity of the macronucleus and that the ultimate unit of mating type determination, the sub-nucleus, was not unstable after it had been determined, i.e., after the macronucleus had been formed. This general explanation for selfing in *P. aurelia* appeared to satisfy most of the observations concerning selfers in this species.

Two further lines of evidence (NANNEY 1953a) were set forth as additional support for the hypothesis of structural inhomogeneity. It was argued that if spontaneously arising unstable macronuclei were composed of smaller macronuclear elements of diverse kinds, then selfers should be induced by the fusion of macronuclei which would ordinarily control different mating types. Selfers were induced by this method and appeared to show all the characteristics of spontaneous selfers. Secondly it was argued that small pieces of a "selfing" macronucleus would be more likely to be homogeneous than large pieces of the same macronucleus; hence, if a macronucleus were allowed to break up into small pieces and if these small pieces were allowed to regenerate (see SONNEBORN 1947, 1950), such regenerated fragments would be more likely to control a pure mating type than would the much larger pieces separated at normal fission. This expectation was experimentally verified (SONNEBORN unpublished; NANNEY unpublished).

Preliminary data (NANNEY and CAUGHEY 1953, and unpublished) indicate that the mechanism of mating type determination in *T. pyriformis* is very similar to that in *P. aurelia* (specifically, the Group A varieties of *P. aurelia*). Some of the characteristics of these similar systems of mating type determination are as follows. 1) The mating types are not determined solely by the genotypes of the cells. 2) The unit of mating type inheritance is the caryonide. 3) The temperature prevailing at the time the new macronuclei are developing has some influence on the probability of origin of a macronucleus controlling a particular mating type. 4) Unstable clones may occur and from such unstable clones diverse mating types may be extracted.

Since mating types in both these forms show caryonidal inheritance, it appears highly probable that nuclear differentiation of a similar nature is involved in the determination of mating types in the two forms. Therefore, any insight into the nature of the macronuclear differences in one form should be pertinent to a discussion of the differences in the other form. In this connection the recently proposed gene-dosage hypothesis for mating type differences in *P. aurelia* (NANNEY 1953a) requires re-examination. The simplest application of this hypothesis held that one mating type was characterized by twice as much genetic material as the other. This interpretation is at least conceivable in a form, such as *P. aurelia*, in which only two mating types exist per variety, but becomes extremely improbable in a form with as many as seven different types per variety. This consideration aside, the gene-dosage hypothesis in any simple form now appears untenable. SONNEBORN (1953) has shown that certain breeding results expected on the basis of the hypothesis are not found. Moreover, it has not been possible to demonstrate any consistent difference in the desoxyribose nucleic acid content of cells of different mating type in *P. aurelia* (GUTHE, TEFANKJIAN and NANNEY unpublished). For these reasons it seems advisable to abandon the gene-dosage hypothesis for *P. aurelia* and to consider as essentially similar the systems of mating type determination in *P. aurelia* and *T. pyriformis*.

The hypothesis of structural inhomogeneity (which was independent of the gene-dosage hypothesis) appeared to offer an opportunity for the analysis of the pattern of segregation in selfing clones. The probability of origin in such clones of a macronucleus containing only one type of sub-nucleus, and hence controlling a single pure mating type, would depend upon the total number of sub-nuclei present, the original proportions of the sub-nuclear types and the manner in which the sub-nuclei were separated at nuclear division. An examination of the segregation pattern was, however, difficult in *P. aurelia* because of a relatively short period of vegetative growth before autogamy intervenes and replaces the old macronucleus. The occurrence of selfing and the apparent absence of autogamy in Tetrahymena suggested that here a detailed analysis of the segregation pattern would be feasible. In attempting to study this pattern of segregation it was observed that starvation of selfing clones results in their becoming stable. These results were not expected on the hypothesis of structural inhomogeneity. A macronucleus composed of stable sub-nuclei of diverse kinds could not be transformed into one with only one sub-nuclear type by starvation unless a severe and undemonstrated intra-nuclear selection were involved. The idea that the sub-nuclei are the units of mating type determination

loses its utility, since the sub-nuclei, like the macronucleus as a whole, would have to remain plastic.

The selfers in *P. aurelia* appear to be essentially like those in Tetrahymena. Recent studies (RUDNYANSKY unpublished) suggest that stabilization of selfing clones in this species may also be accomplished by certain treatments. It is possible, however, that more than a single physical basis for selfing exists, that selfing in *P. aurelia* is due to structural inhomogeneity and selfing in Tetrahymena is due to physiological instability. The simplest interpretation of present data would hold, however, that selfing in both species is due to physiological instability of the macronuclei. A similar proposal has been put forth (BUTZEL 1953) independently and on other grounds for the mating types in *P. aurelia*.

Progressive nuclear differentiation in Tetrahymena

The fact of caryonidal inheritance of mating types in Tetrahymena (NANNEY and CAUGHEY 1953, and unpublished) establishes the macronucleus as the focus of mating type determination in this form. Since cells derived from a single exconjugant may show several different mating types, it is clear that cells with identical genotypes may manifest any of several different phenotypes. Evidence now available (NANNEY and CAUGHEY unpublished) demonstrates that one or more genetic loci are involved in determining the mating type potentialities. A central problem is, therefore, the manner in which one of the various gene-controlled potentialities is established to the exclusion of the others. Although present data are not adequate to provide a completely satisfactory answer to this problem, certain characteristics of the nuclear differentiation may be discussed.

The nuclei from which the new macronuclei are derived undoubtedly possess the full spectrum of potentialities. The time at which an exclusion of potentialities is begun is not known with certainty, but it may be correlated with the time of morphological differentiation, when the presumptive macronuclei begin to enlarge. This morphological nuclear differentiation is certainly under the control of the cytoplasm surrounding the nuclei at this time (NANNEY 1953b), but no good evidence is available to show that the cytoplasm has any specific effect in directing the differentiation of the macronucleus in regard to mating type. In this respect the studied strains of *T. pyriformis* resemble the Group A rather than the Group B varieties of *P. aurelia* (SONNEBORN 1947; NANNEY 1954). During this primary stage of development it is possible to direct to some extent the course of nuclear differentiation by means of temperature (NANNEY and CAUGHEY 1953); the frequencies of the types observed at maturity depends upon the temperature at which conjugation and/or macronuclear development occurs.

The two new macronuclei in an exconjugant are separated at the first post-zygotic cell division. That the nuclei are at least partially differentiated by this time is shown by the fact that the various sub-lines (sub-caryonides) derived from one of these first fission products are strongly correlated in their mating types while the sub-lines derived from diverse caryonides are not correlated. That the macronuclei are not completely differentiated at this time is shown by the fact that several different mating types *may* be derived from a single first fission product. Thus, by the time of the first

post-zygotic cell division the nuclei have passed from an originally totipotent state to one in which the mating type potentialities are severely restricted.

At the time of maturity, many fissions later, over half the nuclei may still possess multiple potentialities but one potentiality is usually predominant. A final exclusion of potentialities may be brought about by the starvation of the cells. The mechanism of this exclusion is not known, but once exclusion is complete the nuclei only rarely, if ever, return to a pluripotent condition. Present data suggest that this final limitation of potentialities is not related to those processes responsible for the maturation of the cells.

SUMMARY

1. Intra-clonal conjugation (selfing) in the WH strains of *Tetrahymena pyriformis* is shown to be due, in many cases at least, to the presence of two or more mating types within a clonal culture.

2. The mating type diversities which are detected in clonal cultures arise during cytologically normal vegetative growth.

3. The cells in such unstable clones may be stabilized, i.e., may lose the capacity to produce several mating types, if they are permitted to starve.

4. This stabilization by starvation can apparently occur over a wide range of time during the growth of the clones, whether or not the cells are mature and whether or not selfing has been detected in the cultures.

5. These observations, in conjunction with others previously reported, are interpreted as indicating a progressive nuclear differentiation whereby the potentialities of a clone are gradually delimited. Certain aspects of this differentiation are under experimental control.

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VARIABLE CROSSING OVER ARISING IN DIFFERENT STRAINS OF DROSOPHILA PSEUDOOBSCURA[1]

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IT HAS been shown (LEVINE and LEVINE 1954) that the amount of crossing over in the X chromosome of *Drosophila pseudoobscura* can be modified by different third chromosome inversion homozygotes and heterozygotes. Thirty-five different combinations of inversion homo- and heterozygotes were found to be capable of a complex genetic interaction giving rise to variable crossover frequencies in the X chromosome. On the basis of this observation an hypothesis of genotypic control of crossing over was proposed to explain, at least in part, the significant variations in crossing over. The hypothesis proceeds from the assumption adumbrated in the work of GOWEN (1919) that crossing over is a character subject to genetic modification. Since the hypothesis was derived from a study of the effect which presumably genetically different third chromosomes had on crossing over in the X chromosome, it was of importance to analyze crossing over in the different third chromosomes themselves. If there is genetic control of crossing over one would predict that analysis of a number of different strains of wild type flies would yield significantly different results for crossing over in any region of a chromosome under consideration.

The present communication utilizing *D. pseudoobscura* third chromosome gene arrangements deals with experiments designed to test the hypothesis of a genetic control of crossing over.

MATERIALS AND METHODS

D. pseudoobscura provides excellent material for an analysis of variation in crossing over since there are a number of strains of third chromosome inversion types available. Since each strain of any given inversion had its origin from a single female caught in nature, it has a great probability of being genetically distinct and might therefore provide a characteristic pattern of crossing over.

The third chromosome strains used in this study were the gene arrangements known as Standard (ST) and Arrowhead (AR). They were obtained from a 1952 collection made by PROFESSOR CARL EPLING and DR. DONALD F. MITCHELL in the San Jacinto Mountains of southern California.

The ST gene arrangement carries the only markers suitable for crossover analysis. These markers are orange eye (*or*), Blade wings (*Bl*), Scute bristles (*Sc*) and purple eye (*pr*). Crossing over between this marked chromosome and five strains of ST and three of AR was analyzed. Three of the ST and two of the AR strains correspond to those used in the crossing over study recently reported (LEVINE and LEVINE 1954).

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The experimental procedure was to cross males of a given third chromosome strain to virgin *or Bl Sc pr* (ST)/lethal females. From each cross at least ten *or Bl Sc pr*/+ + + + females were recovered and mated singly to *or pr* males for the test generation. The parents were then permitted to remain and lay eggs in 25 × 95 mm shell vials containing a corn meal, molasses, agar, and yeast medium for three days whereupon they were transferred to a new vial. This transfer was carried out so that there were five three-day laying periods. Records were kept on each female for each laying period thus permitting an analysis of crossing over as it varied from female to female and variation in crossing over between laying periods. All cultures were maintained at 25°C.

RESULTS

The results illustrating the amount of crossing over for each of the ST third chromosome strains studied are given in table 1. Recorded in the table are the total number of flies counted in each strain for the second through the fifth laying periods. The first laying period has not been included since few and occasionally no eggs were laid. The total number of flies fall into the classes of non-crossovers; single crossovers between *or* and *Bl* (region 1), *Bl* and *Sc* (region 2), and *Sc* and *pr* (region 3); double crossovers in regions 1 and 2, regions 1 and 3, and regions 2 and 3; and triple crossovers in regions 1, 2, and 3. These same data but expressed in terms of the total percent of crossing over for each strain are shown in table 3.

Table 1 shows that the crossing over for the different ST strains is variable. Such variations in crossing over might be due to differential viability of the complementary classes of flies (e.g. + and *or Bl Sc pr*, *or* and *Bl Sc pr*, *or Bl* and *Sc pr*, etc.). In order to test for this possibility a series of homogeneity chi-square tests was carried out. First, a homogeneity chi-square showed no significant variation between females of any strain for a given laying period. Thus, for each strain it was possible to consider the data at a given laying period as the total from all females involved. To further ascertain whether differential viability might account for the observed variation in crossing over between strains a test was made of the homogeneity of the complementary genotypes of each class of flies given in table 1, through all laying periods of the five strains. The chi-square values for 19 degrees of freedom along with their respective P values are given under each class at the bottom of table 1. It is clear from these values that the proportion of the complementary genotypes for a given class is constant between strains. Thus, it is improbable that the differences in crossing over result solely from differential viability. The results obtained with the AR chromosomes are summarized in tables 2 and 3. Data for individual egg laying periods are not presented since so few crossovers occur between the AR and the *or Bl Sc pr* (ST) chromosome.

The nature and significance of the variation in crossing over between the strains can be best demonstrated by an analysis of variance. In this analysis the components of variance are the variations between strains, between the three chromosome regions, and between egg laying periods; and the interactions of strains and chromosomal regions, of strains and laying periods and of chromosomal regions and laying periods.

The analysis of variance of the five ST strains is given in table 4 while that of the

TABLE 1

Crossing over in the or Bl Sc pr *regions of the third chromosome for ST gene arrangements*

Period	0		1		2		3		1-2		1-3		2-3		1-2-3		Total
	+	*or Bl Sc pr*	*or*	*Bl Sc pr*	*or Bl*	*Sc pr*	*or Bl Sc*	*pr*	*or Sc pr*	*Bl*	*or pr*	*Bl Sc*	*or Bl pr*	*Sc*	*or Sc*	*Bl pr*	
ST-1																	
II	276	160	34	35	88	61	109	96	1	1	3	17	12	14	0	0	907
III	230	191	23	28	77	66	100	79	1	1	14	17	7	21	0	0	855
IV	208	116	25	22	70	48	98	80	2	2	10	14	20	24	0	0	739
V	283	155	35	33	103	79	137	107	1	2	15	21	12	29	0	0	1012
Total	997	622	117	118	338	254	444	362	5	6	42	69	51	88	0	0	3513
ST-2																	
II	267	184	17	33	61	46	79	86	1	1	5	10	11	8	0	1	810
III	261	211	27	40	83	76	98	103	0	2	5	8	9	3	0	1	927
IV	274	189	28	28	81	77	103	115	2	0	7	7	13	9	1	0	934
V	358	248	42	35	97	77	137	139	0	0	6	9	15	18	0	1	1182
Total	1160	832	114	136	322	276	417	443	3	3	23	34	48	38	1	3	3853
ST-3																	
II	201	144	27	21	80	41	96	101	1	0	5	10	12	8	0	0	747
III	197	116	20	21	73	59	90	100	1	0	4	3	8	12	0	0	704
IV	252	165	33	24	87	66	117	100	0	1	12	6	14	12	1	0	890
V	238	158	31	33	112	67	120	126	0	0	7	12	19	21	1	0	945
Total	888	583	111	99	352	233	423	427	2	1	28	31	53	53	2	0	3286
ST-4																	
II	208	163	26	26	64	36	60	63	0	1	5	4	8	10	0	0	674
III	313	232	43	35	78	66	110	99	0	1	7	9	5	10	0	0	1008
IV	275	166	27	25	57	62	91	82	0	2	6	7	8	10	1	0	819
V	235	144	32	31	61	68	94	73	1	0	9	6	7	13	0	0	774
Total	1031	705	128	117	260	232	355	317	1	4	27	26	28	43	1	0	3275
ST-5																	
II	169	122	21	15	54	49	77	79	0	0	3	6	6	15	0	0	616
III	249	148	38	22	86	71	101	113	0	2	6	13	11	17	0	0	877
IV	202	138	27	15	71	62	102	96	1	0	7	9	5	4	1	0	740
V	164	102	20	15	38	36	58	65	0	0	7	9	8	10	0	0	532
Total	784	510	106	67	249	218	338	353	1	2	23	37	30	46	1	0	2765
χ^2	24.49		20.60		22.63		16.24				12.29		26.00				
P	0.17		0.35		0.25		0.65				0.85		0.16				

TABLE 2

Crossing over in the or Bl Sc pr *regions of the third chromosome for AR gene arrangements*

Period	0		1		2		3		1-2		1-3		2-3		1-2-3		Total
	+	*or Bl Sc pr*	*or*	*Bl Sc pr*	*or Bl*	*Sc pr*	*or Bl Sc*	*pr*	*or Sc pr*	*Bl*	*or pr*	*Bl Sc*	*or Bl pr*	*Sc*	*or Sc*	*Bl pr*	
AR-1 II–V	1426	980	30	24	7	6	0	13	0	0	0	0	0	0	0	0	2486
AR-2 II–V	1839	1596	17	12	4	3	0	8	0	0	0	0	1	0	0	0	3480
AR-3 II–V	2136	1870	54	70	20	16	0	10	1	0	0	0	2	0	0	0	4179

TABLE 3

Percent crossing over by regions

	ST-1	ST-2	ST-3	ST-4	ST-5	Mean	AR-1	AR-2	AR-3	Mean
or-Bl	10.2	8.2	8.3	9.3	8.6	8.9	2.17	0.83	2.99	2.00
Bl-Sc	21.1	18.0	21.2	17.4	19.8	19.5	0.52	0.23	0.93	0.56
Sc-pr	30.1	26.1	30.9	24.3	30.0	28.3	0.52	0.26	0.29	0.36

TABLE 4

Analysis of variance of crossing over in ST chromosomes

	ss	*df*	*ms*	*F*	*P*
Strains (S)	18,474.10	4	4,618.525	17.52	<0.001
Regions (R)	259,326.43	2	129,663.215	491.76	<0.001
Periods (P)	11,624.33	3	3,874.777	14.70	<0.001
S × R	5,206.40	8	650.800	2.47	0.01-0.05
S × P	25,380.17	12	2,115.014	8.02	<0.001
R × P	2,454.37	6	409.062	1.55	>0.05
Remainder	6,328.13	24	263.672	—	—
Total	328,793.93	59			

TABLE 5

Analysis of variance of crossing over in AR chromosomes

	ss	*df*	*ms*	*F*	*P*
Strains (S)	757.56	2	378.780	40.01	<0.001
Regions (R)	1,468.23	2	734.115	77.54	<0.001
Periods (P)	216.11	3	72.037	7.61	0.007
S × R	623.27	4	155.818	16.46	<0.001
S × P	64.22	6	10.703	1.13	>0.10
R × P	173.55	6	28.925	3.06	0.05
Remainder	113.62	12	9.468	—	—
Total	3,416.56	35			

AR strains is shown in table 5. Considering the ST strains first it is seen that the main effects (those of strains, regions, and periods) are significant. Thus, as shown in table 3, the five strains produce significantly different amounts of crossing over in the *or Bl Sc pr* region of the third chromosome. In addition the total amount of crossing over is significantly different between the four laying periods. Each strain, therefore, has its characteristic pattern from period to period. The significant P value for regions is to be expected since the four loci under consideration are widely separated along the length of the chromosome.

The interactions are of particular interest in that they show that differences in crossing over need not arise merely in the total amount of crossing over between the five strains. The interaction of strains and regions is significant. Thus, the strains may not respond similarly in their total percent crossing over for these specific third chromosome regions.

The second interaction, that of strains and periods, is highly significant and indicates that the strains respond differently during the four laying periods. The interaction of regions and periods is not significant; therefore the regions as a whole regardless of the strain have the same mean percent crossing over at each laying period.

The analysis of variance for the three AR strains as presented in table 5 is substantially the same as the ST analysis. The variations between strains, regions, and periods all contribute significantly to the variance as does the interaction of strains and regions. However, the interaction of strains and periods is not significant and the regions by periods interaction is just significant at the 5 percent level. As was the case with ST chromosomes, different AR chromosomes behave differently with respect to the amount of crossing over per region. However, the strains do not behave differently with respect to the several laying periods. There is variation in the regions with the laying period which was not present in the ST strains. Unfortunately, the very small number of crossovers produced in the AR/*or Bl Sc pr* (ST) heterozygotes makes interpretation of the interactions difficult. In general it may be said that the three strains differ from each other markedly in their amount of crossing over and that there is a significant effect of the laying period on crossing over.

DISCUSSION

Crossing over between an *or Bl Sc pr* (ST) third chromosome and five strains of ST chromosomes of natural origin results in significant variations. The analysis of variance shows that the strains differ in their total percent of crossing over. Secondly, differences in crossing over are exhibited by the strains for the *or-Bl*, *Bl-Sc*, and *Sc-pr* regions of the chromosome. Thus it would appear that each third chromosome strain determines to some extent its percent of crossing over. In addition, a significant difference arises between the five egg laying periods and in the interaction between strains and egg laying periods. Accordingly, it may be assumed that the age of the female has an effect on crossing over. Though BRIDGES (1915) reports an effect of age on crossing over in *D. melanogaster* it is of a distinctly different character from that observed here for *D. pseudoobscura*. BRIDGES' data showed that third chromosome crossing over underwent a marked drop in the first nine days of the female's egg laying followed by a gradual rise to a level never exceeding the initial peak.

The most distinct changes arose between loci just on either side of the centromere. In the case of *D. pseudoobscura* no such pattern appears among the five ST strains. This may result from a fundamental difference between the two species or possibly from the fact that the centromere region only is sensitive to age. If the latter is true it must be pointed out that the third chromosome of *D. pseudoobscura* has an almost terminal centromere, similar in this respect to the X chromosome of *D. melanogaster*. The effect of age on crossing over in general is not clear and it is quite possible that the age of the female may have little to do with crossover variations but that variation is due to differences in culture and other external conditions. Further study of the change in crossover percent in succeeding egg laying periods is clearly indicated. However, for purposes of the present study it is sufficient to state that there are significant variations in crossing over as shown by the interaction of strains and egg laying periods and suggest a dependence of crossing over upon the different strains themselves.

The results obtained with the three AR strains agree in general with the ST strains. The major departures arise among the interactions, but as has been pointed out the relatively low number of crossovers makes interpretations difficult.

The major assumption underlying this and our previous study of the genetic control of crossing over is that the third chromosome strains analyzed are genetically diverse. Though it is impossible to demonstrate conclusively, at least by ordinary genetic tests, that different wild type chromosomes have varied genetic constitutions there is indirect evidence from a number of sources. WALLACE, KING, MADDEN, KAUFMANN, and MCGUNNIGLE (1953) have shown in *D. melanogaster* that five wild type third chromosomes isolated from different flies and then permitted to recombine in 55 different ways produced significantly different phenotypes in terms of viability, fertility, and bristle number. Experiments with numerous heterozygous lethals in *D. melanogaster* (STERN, CARSON, KINST, NOVITSKI, and UPHOFF 1952) and *D. willistoni* (CORDEIRO 1952 and CORDEIRO and DOBZHANSKY 1954) have indicated that the lethal heterozygotes can have wide variations in viability. The *D. willistoni* studies in particular have shown that various lethal chromosomes in combination with a number of wild type chromosomes have different viabilities. Though only casual observations have been made, it has been noted that there are marked differences in the fertility, fecundity, and growth rate in the stocks of the *D. pseudoobscura* third chromosome strains as they are maintained in this laboratory.

Experiments on selection for quantitative traits in *D. melanogaster*, in which it has been demonstrated that wild type chromosomes possess polygenic systems controlling such phenotypes as bristle number (MATHER 1943) and body proportions (ROBERTSON and REEVE 1952), have shown that there is genetic variability between wild type chromosomes. It appears certain, therefore, that different but still supposedly wild type chromosomes produce measurably different phenotypes at least where quantitative traits are concerned. There seems to be no reason, therefore, why crossing over should not likewise be quantitatively different in different wild type chromosomes. Actual demonstration of genetic systems controlling crossing over awaits detailed experimentation on selection for rates of crossing over. The work of DETLEFSEN and ROBERTS (1921) is suggestive in this regard but more precise experiments are desirable.

In lieu of evidence to the contrary, the conclusion to be derived from the present study is that the variation in crossing over for the different ST and AR strains results most probably from differences in their genetic constitutions, and thus is in line with our previously reported hypothesis, that crossing over is at least partially under genetic control.

On the basis of this study we predict that chromosomes isolated from different natural populations of Drosophila are in general endowed with genetic factors which will result in differences in crossing over. An analysis of crossing over in individual chromosomes derived from four natural populations of *D. pseudoobscura* is underway in order to test the extent to which this prediction seems to be true.

SUMMARY

Five strains of the ST and three of the AR third chromosome arrangements of *D. pseudoobscura* obtained from different females caught in nature were analyzed for crossing over with an *or Bl Sc pr* (ST) chromosome. Analysis of the results with the five ST strains has shown the following:

1. The strains differ significantly in their total percent of crossing over.
2. There are significantly different amounts of crossing over in successive egg laying periods and, in addition, each strain has its own pattern of crossing over from period to period.
3. Significant differences in crossing over are exhibited by the strains within the *or-Bl*, *Bl-Sc*, and *Sc-pr* regions of the chromosome.

The results obtained with the three AR strains agree in general with those found for the ST strains.

It is concluded that the variation in crossing over for different ST and AR strains results, in part, from differences in their genetic constitutions. The results are consistent with an hypothesis which suggests that crossing over is at least partially under genetic control.

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PROGENY OF ARTIFICIALLY INDUCED SEX-REVERSALS OF MALE GENOTYPE (XY) IN THE MEDAKA (ORYZIAS LATIPES) WITH SPECIAL REFERENCE TO YY-MALE

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THE genetic mechanism for sex determination in the medaka (*Oryzias latipes*), where the female is homogametic (XX) and the male heterogametic (XY), was established by AIDA (1921). There are a number of color varieties in the medaka. According to AIDA (1921) the various color effects are referred to the action of genes, as indicated by the following formulae when homozygous: brown (wild) *BBRR*, orange-red (called red) *bbRR*, white *bbrr*, variegated orange-red *B'B'RR*, and variegated white *B'B'rr*. The multiple alleles *B*, *B'* and *b* control melanin formation and are autosomal. The alleles *R* and *r* control the formation of orange-red pigment and are linked to the X or Y chromosomes or both.

Since both white (*bbrr*) and red (*bbRR* or *bbRr*) medakas have common recessive genes *bb*, their phenotypes (*br* and *bR*) may be expressed simply as *r* and *R*, respectively, when reference is made to these two varieties only.

When a white female (X^rX^r) is crossed with a heterozygous red male (X^rY^R), the offspring are white females and red males in equal numbers because in this mating system the dominant red gene *R* exists only in the Y chromosome. The father-to-son inheritance of the red condition *R*, was confirmed in my breeding experiments in three consecutive generations by crossing white females (X^rX^r) with heterozygous red males (X^rY^R). Therefore the sex-linked characters *R* and *r* can be used as sex-chromosomal "markers". Previous study has established that the oral administration of estrogens (estrone or stilbestrol) at the time of hatching and for several months thereafter induced complete sex-reversal in the genotypic male (X^rY^R).

All the red (*R*) fish developed into perfect females, and when fully grown these sex-reversals have the functional sex-equipment and reproductive capacity of the female. It was concluded that complete and functional sex-reversal from male to female can be artificially induced under the influence of estrogens (YAMAMOTO 1953).

A genetic study on the offspring of sex-reversals of the male genotype affords us further proof of the genetic constitution of sex-reversals. When sex-reversed XY females are mated with normal XY males, their offspring should be XX females, normal XY males and YY males in the 1:2:1 ratio if YY males are viable. Can YY males be found among F_1 offspring of induced sex-reversals of the male genotype? If this question is answered in the affirmative, we are then provided with further proof that we are correct in the conclusion that sex-reversals induced by estrogens had the sex genotype of the male (XY).

MATERIALS AND METHODS

Genetic analyses were made of two fully grown red (X^rY^R) females, E8 and E9 showing sex-reversal under the action of estrone and two fully grown red (X^rY^R) females, S8 and S9 showing sex-reversal through the influence of stilbestrol, designated as estrone-sex-reversed females and stilbesterol-sex-reversed females, respectively. The two estrone-sex-reversed females were mated with two normal red males (X^rY^R) and the two stilbestrol-sex-reversed females were mated with two normal red males (X^rY^R). The F_1 offspring of sex-reversals were tested by fish of known genotypes. Fish used as testers were derived from consecutive backcrossings between white females (X^rX^r) and heterozygous red males (X^rY^R) and were fifth and sixth generation. These generations are designated as FR_5 and FR_6 (R = backcross to recessive), respectively. Results of progeny testing were examined when fish reached maturity or an age at which the sex could be determined by external sexual characteristics. External determination of the two sexes is easily possible by their secondary sexual characteristics (*cf.* OKA 1931, OKADA and YAMASHITA 1944, YAMAMOTO 1953).

As the medaka is an oviparous cyprinodont, fertilization and development are normally external. In the breeding season, a mature female may produce a batch of eggs daily for a considerable period of time. That internal fertilization may take place in rare cases and that development in the ovarian cavity of the parent may proceed in some degree, was pointed out by AMEMIYA and MURAYAMA (1931). In the present study every care was taken to guard against this possibility when previously mated females were used. The first hatched larvae were usually discarded to avoid a possible mixture of zygotes in which some would have been previously fertilized by an unknown father.

Crossings were carried out indoors in glass jars which contained water hyacinths (*Eichhornia crassipes*). Their long, dense roots are admirable as egg receivers. Shortly before hatching time, water hyacinths, with eggs attached, were removed from the jar-aquaria to outdoor concrete pools measuring 75 cm. square and 18 cm. deep, remaining there until they attained maturity. The fry were given a standard dried food composed of the following ingredients: 60 g. powdered shrimp, 30 g. toasted wheat grains pounded to flour (Japanese "kosen"), 6 g. yeast preparation ("wakamoto") and 4 g. powdered green tea ("macha"). From the young to the adult stage their diet consisted chiefly of a mixture of powdered shrimp and toasted wheat-flour (kosen) to which we occasionally added water fleas (*Moina macrocopa*) and tubifeciid worms (*Limnodrilus* and others).

All of our body length measurements were made on fish in an anesthetized state and were taken from the tip of the snout to the end of the caudal fin.

F_1 OFFSPRING OF ESTRONIZED AND STILBESTROLIZED FEMALES OF FEMALE GENOTYPE

Before proceeding further, a brief account is given of the F_1 offspring of estrogen-administered genotypic females (X^rX^r). These white females were mated with FR_4 red males of the genotype X^rY^R. As noted in the previous paper, they were proved to be fertile. Three estronized white females (X^rX^r) in mating with normal red

males (X^rY^R) produced 60 white (*r*) daughters, 76 red (*R*) sons and one exceptional red (*R*) daughter. This exceptional daughter was tested by crossing with a red male (X^rY^R) but the offspring failed to survive. A stilbestrolized white female (X^rX^r) mated to a normal red male (X^rY^R), produced a few offspring, of which only two white (*r*) daughters matured.

F_1 OFFSPRING OF INDUCED SEX-REVERSALS

The F_1 offspring produced by mating sex-reversed red (X^rY^R) females with normal heterozygous red (X^rY^R) males were fully grown by the summer of 1952. Based upon the theoretical diagram of the F_1 offspring of sex-reversals, as shown in figure 1, we could expect that phenotypically the F_1 offspring would include white (*r*) females and red (*R*) males in the ratio of 1:3, and genotypically white females (X^rX^r), ordinary red males (X^rY^R) and extraordinary red males (Y^RY^R) in the ratio of 1:2:1, if YY zygotes could develop into mature males. Observed F_1 offspring of estrone-sex-reversals and of stilbestrol-sex-reversals are shown in table 1. Out of 74 F_1 offspring of estrone-sex reversals, 21 were white daughters and 51 were red sons with one exceptional white son and one exceptional red daughter. Observed ratio of white females to red males was 1:2.4 while theoretically it is 1:3. Out of 177 F_1 offspring of stilbestrol-sex-reversed females 54 were white daughters and 119 were red sons with three exceptional white sons and one exceptional red daughter. Observed ratio of white females to red males was 1:2.2 instead of the theoretical 1:3 ratio.

By a preponderance of males, the conclusion set forth in the previous paper (YAMAMOTO 1953) is verified, i.e., that sex-reversals must have the genetic constitution X^rY^R. If they had the genetic constitution X^rX^R, as the result of either crossing over between the X^r and Y^R chromosomes in their male parent or mutation from X^r to X^R, the sex-ratio of the F_1 offspring would be 1:1 and white females (*r*♀), red females (*R*♀) and red males (*R*♂) would be produced in the ratio of 1:1:2. The observed ratio (table 1) definitely differs from the assumption that they have the

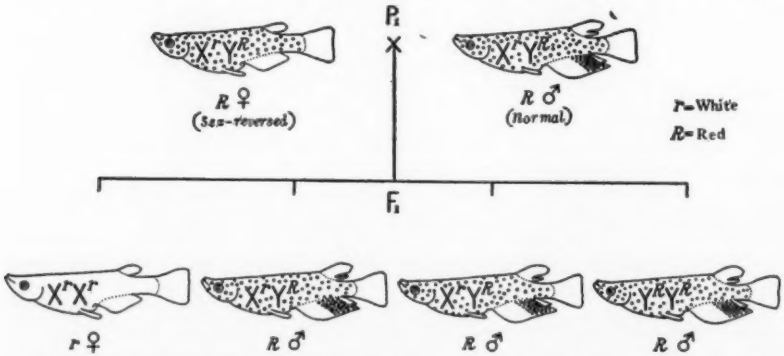

FIGURE 1. Theoretical diagram of F_1 offspring produced by mating a sex-reversed female of the male genotype (X^rY^R) with a normal male (X^rY^R).

TABLE 1

F_1 offspring from estrone- and stilbestrol-treated red females of male genotype (X^rY^R) mated with FR_4 normal red males (X^rY^R)

	Estrone treated		Stilbestrol treated	
	Observed	Expected	Observed	Expected
White females	21	18.5	54	44.3
White males	1	0	3	0
Red females	1	0	1	0
Red males	51	55.5	119	132.7
Total	74	74.0	177	177.0

genetic constitution X^rX^R but it coincides with the assumption that they have the genetic constitution X^rY^R if we assume that Y^RY^R zygotes were fewer in number than the expected value.

GENETIC ANALYSIS OF EXCEPTIONAL WHITE MALES AND EXCEPTIONAL RED FEMALES AMONG F_1 OFFSPRING OF SEX-REVERSALS OF MALE GENOTYPE

Genetic analyses of exceptional white sons and exceptional red daughters are given before passing on to the results of progeny tests of F_1 red sons. One exceptional white son (labeled No. 74) and one exceptional red daughter (No. 73) were yielded among F_1 offspring of estrone-sex-reversed females (table 1). Three exceptional white sons (No. 174, 175, 176) and one exceptional red daughter (No. 177) appeared among F_1 offspring of stilbestrol-sex-reversed females (table 1).

There are three possibilities for the production of exceptional white sons and exceptional red daughters. The first possibility is that they may be the result of crossing over between the X^r and Y^R chromosomes, which produce the X^R and Y^r gametes. If fertilization occurs between the X^R and X^r gametes, or between the Y^r and X^r gametes, both a red daughter (X^rX^R) and a white son (X^rY^r) are produced. A second possibility is that these exceptional fish may be the product of mutation of genes, X^r to X^R or Y^R to Y^r. The third possibility is that an exceptional white son may be a spontaneous sex-reversal of the female genotype (X^rX^r) and an exceptional red daughter may be a spontaneous sex-reversal of the male genotype (X^rY^R).

Progeny tests of exceptionals were explored to determine which possibility is correct. The results are summarized in table 2. An exceptional white son, $F_1(E)r$ No. 74, among F_1 offspring of estrone-sex-reversals was mated with white females of the genetic constitution X^rX^r, with the result that white females and white males were approximately equal numbers among the progeny. If the genetic constitution of $F_1(E)r$ No. 74 had been X^rX^r, an all-female broods could have been expected. The result indicates that the exceptional white son has the genetic constitution X^rY^r. An exceptional red daughter, $F_1(E)R$ No. 73, among F_1 offspring of sex-reversals was mated with a heterozygous red male, FR_5 $R\sigma^{\prime}$, pedigreed genetic constitution of which was X^rY^R. Among the offspring of this cross, white females, red females and red males in the 1:1:2 ratio were obtained. Had the exceptional female been a

TABLE 2

Progeny tests of exceptional white (r) *males and exceptional red* (R) *females among F_1 offspring of sex-reversed X^rY^R females.* r = *white*, R = *red*

Expt. No.	Parents		Offspring				Genotype of exceptional fish
	♀	♂	*r*♀	*r*♂	*R*♀	*R*♂	
51	$r(X^rX^r)$	$F_1(E)r$ No. 74	20	14	0	0	X^rY^r
52	$F_1(E)R$ No. 73	$FR_5\ R(X^rY^R)$	6	0	7	11	X^rX^R
151	$F_1(S)R$ No. 177	$FR_5\ R(X^rY^R)$	—	—	—	—	—
152	$r(X^rX^r)$	$F_1(S)r$ No. 174	17	22	0	0	X^rY^r
153	$r(X^rX^r)$	$F_1(S)r$ No. 175	9	8	0	0	X^rY^r
154	$r(X^rX^r)$	$F_1(S)r$ No. 176	65	72	0	0	X^rY^r

spontaneous sex-reversal of the male gentoype X^rY^R, then white females and red males in the 1:3 ratio could have been expected and no red females would have occurred. The result indicates that the genetic constitution of the exceptional red daughter was X^rX^R, and that white females (X^rX^r), red females (X^rX^R) and red males (X^rY^R and X^RY^R) in the ratio of 1:1:2 were produced.

To test the genotype of an exceptional red daughter, $F_1(S)R$ No. 177, among F_1 offspring of stilbestrol-sex-reversals of the male genotype, she was mated with a heterozygous red male ($FR_5\ R♂$) of the genetic constitution X^rY^R. Unfortunately she died before she could produce offspring. Exceptional white sons, $F_1(S)r$ No. 174, $F_1(S)r$ No. 175 and $F_1(S)r$ No. 176 among F_1 offspring of stilbestrol-sex-reversals of the male genotype were singly mated with white females, $FR_5\ r♀$, of the constitution X^rX^r. Their offspring were equally white females, and white males. If the exceptional white sons had been spontaneous sex-reversals of the female genotype (X^rX^r), an all-female broods could have been expected. Progeny tests proved that the exceptional white sons had the genetic constitution X^rY^r.

These results indicate that these exceptionals among F_1 offspring of sex-reversals of the male genotype had the genetic constitution either X^rY^r or X^rX^R. This may have resulted from either crossing over between the X^r and Y^R chromosomes or mutations of genes, X^r to X^R and Y^R to Y^r. Judging from the frequency of exceptionals the first alternative appears the most plausible.

PROGENY TESTS OF RED SONS OF SEX-REVERSALS OF MALE GENOTYPE

Table 1 indicates that 51 red sons were produced among F_1 offspring of estrone-sex-reversals and 119 red sons were produced among F_1 offspring of stilbestrol-sex-reversals. Since both parents have the genetic constitution X^rY^R, it might be expected that the ratio of white daughters and red sons would be 1:3, and also that red sons would include X^rY^R and Y^RY^R males in the 2:1 ratio if Y^RY^R zygotes are able to develop to maturity. As mentioned before, the observed ratio of white females to red males was 1:2.4 among F_1 offspring of estrone-sex-reversed females and 1:2.2 among F_1 offspring of stilbestrol-sex-reversed females instead of the theoretical 1:3. This discrepancy suggests the rarity of Y^RY^R males. Since the two genotypes of red males, X^rY^R and Y^RY^R, can not be identified phenotypically,

TABLE 3

Progeny tests of F_1 red sons of estrone- and stilbestrol-sex-reversed females of male genotype (X^rY^R). Sons of estrone-reversed females are designated by $F_1(E)R$; of stilbestrol-reversed females by $F_1(S)R$. The total offspring of all matings that yielded offspring of both sexes are pooled

Number of matings	Parents		Offspring				Genotype of ♂ parent
	♀	♂	r♀	r♂	R♀	R♂	
19	$r(X^rX^r)$	$F_1(E)R$	360	1	4	420	X^rY^R
1	$r(X^rX^r)$	$F_1(E)R$	0	0	0	72	Y^RY^R
21	$r(X^rX^r)$	$F_1(S)R$	404	0	12	410	X^rY^R

progeny tests were performed to determine their genotypes. If a red son of the genotype X^rY^R is mated to white females (X^rX^r), 50 percent of his offspring would be white females and 50 percent would be red males. If a red son of the genotype Y^RY^R is mated to white females (X^rX^r), all of his offspring would be red males.

Red sons produced by mating estrone-sex-reversed females with normal red males were designated as $F_1(E)R$♂ and red sons from matings of stilbestrol-sex-reversed females and normal red males were labeled $F_1(S)R$♂. Twenty-two $F_1(E)R$ males and twenty-eight $F_1(S)R$ males were chosen at random and submitted to progeny tests by crossing with white females (X^rX^r). Of twenty-two $F_1(E)R$ males tested two died before producing offspring. Of twenty-eight $F_1(S)R$ males tested seven died before yielding offspring. Therefore, only twenty $F_1(E)R$ males and twenty-one $F_1(S)R$ males successfully produced offspring which belonged to the F_2 generation of sex-reversals.

When the offspring were fully grown, they were examined as to sex and phenotypes. The results are shown in table 3. Table 3 indicates that with one exception $F_1(E)R$ males in crosses with white females (X^rX^r) produced white females and red males in approximately equal numbers, indicating that the genotype of the male parent was X^rY^R. One test, however, yielded 72 mature offspring, all of which were red males. This clearly shows that the genotype of this male parent, which was a red son of an estrone-sex-reversed mother was Y^RY^R and the genotype of his offspring was presumably X^rY^R. As is also shown in table 3, all twenty-one $F_1(S)R$ males tested produced white females and red males. All produced nearly equal numbers except one which produced 8 females and 23 males. The genotype of male parents tested must be X^rY^R. No Y^RY^R male was detected among red sons of stilbestrol-sex-reversals. Since only twenty-one out of 119 red sons were successfully submitted to test mating, there remains a possibility that some Y^RY^R males may be among non-tested red sons. Judging from the ratio of 1 white female to 2.2 red males and not of 1:3 among F_1 offspring of the stilbestrol-sex-reversals (table 1), it is expected that Y^RY^R males would be but few among $F_1(S)R$ males.

PROGENY TESTS OF EXCEPTIONALS AMONG F_2 OFFSPRING OF SEX-REVERSALS

A few exceptional red females and one exceptional white male were produced in progeny tests of the red sons of induced sex-reversals (table 3). In the summer of 1953 fifteen exceptionals were tested to determine their genotypes. Exceptional red

TABLE 4

Progeny tests of exceptional red females and an exceptional white male among F_2 offspring of sex-reversals of the male genotype (cf. *table 3*). r = *white*, R = *red*

Expt. No.	Parents		Offspring				Genotype of except. fish
	♀	♂	*r*♀	*r*♂	*R*♀	*R*♂	
Ex-2	*R*(104-ex-1)	*R*(X^rY^R)	59	0	2	127	X^rY^R
Ex-3	*R*(104-ex-2)	*R*(X^rY^R)	34	1	0	57	X^rY^R
Ex-6	*R*(105-ex-1)	*R*(X^rY^R)	9	0	7	13	X^rX^R
Ex-15	*r*(X^rX^r)	*r*(14-ex-1)	28	33	0	0	X^rY^r

females were singly tested by crossing with red males (X^rY^R) and an exceptional white male was tested by mating with a white female (X^rX^r). Eleven of the fifteen tested either failed to produce mature offspring or those obtained were not sufficient to determine their genotypes. Only four exceptionals produced a sufficient number of offspring to decide their genetic constitutions, the result of which are summarized in table 4.

Two exceptional red females *R*(104-ex-1) and *R*(104-ex-2) singly mated to X^rY^R males produced white females and red males in the 1:2 ratio with two exceptional red females and one exceptional white male. These two exceptional red females which were tested might be spontaneous sex-reversals of the male genotype (X^rY^R). If they had the genetic constitution X^rY^R and were mated to X^rY^R males, the expected offspring would be phenotypically white females (*r*♀) and red males (*R*♂) in the 1:3 ratio, and genotypically white females (X^rX^r), ordinary red males (X^rY^R) and extraordinary red males (Y^RY^R) in the ratio of 1:2:1. The discrepancy between expected 1:3 and observed 1:2 ratios may be attributed to the rarity of Y^RY^R males.

One exceptional red female *R*(105-ex-1) among F_2 offspring of induced sex-reversals in mating with a red male (X^rY^R) produced white females, red females and red males in the ratio of approximately 1:1:2, showing she was of the genetic constitution X^rX^R as a result of crossing over. One exceptional white male *r*(14-ex-1) among F_2 offspring of induced sex-reversals mated with white females (X^rX^r), fathered white females and white males in the 1:1 ratio indicating that he was of the genetic constitution X^rY^r. This exceptional white male is accounted for by crossing over in the sex chromosomes of the male parent.

Table 3 indicates a preponderance of exceptional red females over exceptional white males. This is attributed to the fact that exceptional red females among F_2 offspring of induced sex-reversals include both X^rX^R and X^rY^R females resulting from crossing over and spontaneous sex-reversal of the male genotypte, respectively.

F_3 AND F_4 OFFSPRING OF INDUCED SEX-REVERSALS

The offspring of exceptionals among F_2 progeny of induced sex-reversals have already been described (table 4). These offspring belong to the F_3 generation of sex-reversals.

In the summer of 1953, using F_2 offspring of induced sex-reversals, breeding experiments were also performed with normal white females (X^rX^r) and normal red males

(X^rY^R). F_2 white females and F_2 red males of estrone-sex-reversals were inbred. The same breeding experiment was performed with the F_2 offspring of stilbestrol-sex-reversals. Matings of white females (X^rX^r) and red males (X^rY^R) among F_2 offspring of estrone-sex-reversals produced 17 white females and 28 red males. Crossings of white females (X^rX^r) and red males (X^rY^R) among F_2 offspring of stilbestrol-sex-reversals yielded 13 white females and 17 red males. These F_3 white females and red males matured by the summer of 1954 and they produced a vast number of young which are thriving normally in our outdoor concrete pools.

SECONDARY SEXUAL CHARACTERISTICS IN YY MALE

As previously mentioned, a single YY male, $F_1(E)R$ No. 19, was detected among F_1 offspring produced by mating estrone-sex-reversals of the male genotype (X^rY^R) with normal red males (X^rY^R). This male, after it had produced a considerable number of the offspring in cross with white females (X^rX^r) and before it was proved to be YY male by its unisexual male progeny, was fixed with formalin. Figure 2 is a photograph of this male. Its general physical characteristics were similar to those of ordinary (XY) males. Secondary characteristics of the YY male were studied. The most prominent external sexual characteristic *inter alia* in the male of the medaka is the presence of numerous numbers of papillar processes on the anal fin, according to OKA (1931). Papillar processes first appear when the male reaches a length of 22–26 mm (caudal fin included) and they increase in number with age. Usually they are present in pairs on segments of several posterior anal fin-rays. NAGATA (1934, 1936) and OKADA and YAMASHITA (1944) experimentally proved that they are manifestations of the male hormone and OKADA (1952) pointed out that they can be used as a quantitative determination of androgens. Therefore, the total number of papillar processes on the anal fin may be regarded as an outward sign of functional activity of the testis. Counts were made of both numbers of anal fin-rays which carry the processes and the total number of the processes in the YY male and in the ordinary (XY) males, the genotype of which have been determined

FIGURE 2. A YY male of the medaka (*Oryzias latipes*). An orange-red son, $F_1(E)R$ ♂ No. 19, detected among F_1 offspring produced by mating estrone-sex-reversed females of the male genotype (X^rY^R) with normal heterozygous orange-red males (X^rY^R). This Y^RY^R male in cross with white females (X^rX^r) fathered all-male broods, a total of 72 orange-red sons.

TABLE 5

Papillar processes (P.P.) on anal fin-rays of normal (XY) males and the YY male. Figures in the first row are average values. Figures in parentheses are ranges of variability. s = Standard deviation. V_c = Coefficient of variability

Genotype of males	Number of fish	Body length in mm.	Number of anal fin-rays	Numb. of P.P.-bearing anal fin-rays	Total numb. of P.P.
X^rY^R	32	36.6 ± 0.3 (31.5 − 42.0) s = ±2.5 V_c = 6.7	20 ± 0.2 (18 − 23) s = ±1.1 V_c = 5.5	8.7 ± 0.1 (6 − 10) s = ±1.0 V_c = 11.5	280 ± 5 (205 − 357) s = ±38 V_c = 13.6
Y^RY^R	1	36.5	20	10	330

from their progenies. The results are tabulated in table 5. There were more papillar processes in the YY male than the average value plus standard deviation of those in XY males. Although there were some XY males which carry more numerous papillar processes, he was a decidedly masculine male. Since this YY male produced very many offspring, his sexual behavior was also supposed to be vigorous.

DISCUSSION

Fish geneticists have often found spontaneous sex-reversals in their breeding experiments. In certain varieties of the guppy (*Lebistes reticulatus*) where the female is homogametic (XX) and the male heterogametic (XY), WINGE (1930) found XX males. By various systems of selective mating, WINGE (1934) obtained XY females and XX males. He was able to produce YY males through mating exceptional XY females to normal XY males.

Among "domesticated" platyfish (*Platypoecilus maculatus*) where the female is heterogametic (WZ) and the male homogametic (ZZ), BREIDER (1942) found an exceptional male of the female genotype (WZ). The mating of this exceptional WZ male to a normal (WZ) female yielded the offspring in the ratio of 1WW:2WZ:1ZZ, or three females to one male. The WW females were identified by their phenotypes. Unfortunately, however, the genetical behavior of the WW females was not analyzed. If WW females had been mated with ordinary ZZ males, all of their offspring would necessarily have been females. BELLAMY and QUEAL (1951) described a series of mating of the platyfish (WZ-ZZ type). They found exceptional males with reference to their phenotypes. Progeny testings of these exceptionals indicated that some exceptional males were ZZ males produced by crossing over of color genes while the others were either sterile or spontaneously sex-reversed males of the female genotype (WZ). Matings of these exceptional WZ males to normal females (WZ) produced the sex ratio of 3 females to 1 male as in BREIDER's study. About one third of the females when mated with normal male (ZZ) breed as though they were WW and mothered all-female broods. GORDON (1946a, 1946b, 1947) reported a case of spontaneous sex-reversal from female to male direction in "wild" platyfish from the Rio Jamapa (Mexico) population, the genetic constitution of which was XX for female and XY for male. GORDON found an exceptional male of the female genotype (XX). When this

exceptional XX male was mated to a normal "wild" XX female, all their offspring were females. Spontaneous sex-reversal in the platyfish from male to female direction has not yet been found.

AIDA (1936) reported spontaneous sex-reversals in the medaka, *Oryzias* (*Aplocheilus*)*latipes*, where the female is XX and the male XY. In his strain he found a number of exceptional males of the female genotype (XX). According to his opinion this was due to the low potency of the X chromosome caused by many years' close inbreeding. He also reported two cases of spontaneously sex-reversed females of the male genotype (XY). When these exceptional XY females were mated with normal males (XY), they produced the offspring in the sex ratio of 1 female to 3 males. One out of the three males tested was found to be YY male which sired all-male broods. He reported the rarity of spontaneous sex-reversal from male to female direction in his strain.

Accidental hermaphrodites are frequently found in adult frogs. CREW (1921) described a case in which a female frog (*Rana temporaria*) was assumed to have undergone a process of masculinization transforming into a hermaphrodite with predominant male characteristics. This frog in mating with a normal female functioned as a male and sired an all-female brood, as would be expected if it was a genetic female of the constitution XX. WITSCHI (1923) reported a sporadic hermaphrodite in the same form which developed both functional sperm and ova and produced offspring.

Among the vast numbers of sex-reversals known in birds, only one sporadic case of functional sex-inversion has been reported. CREW (1923) described this case of accidental sex-reversal in which a hen became a cock and fathered two chickens. A post-mortem examination of the abnormal fowl revealed that the ovary had been completely destroyed by tuberculosis. This situation has not been duplicated by experimental means. Sex-reversals in fowls induced by sinistral ovariotomy, rarely exhibit spermatogenesis in the right gonad. DOMM (1930) attempted a breeding experiment with such an inverted ovariotomized fowl but no fertility was found.

The cases before mentioned of functional sex-inversion in fish and frogs are spontaneous sex-reversals in normal breeding experiments and not the result of artificial induction. Despite the studies of many investigators on experimental control of sex differentiation in a series of vertebrates, few breeding experiments with sex-reversals thus produced have been reported. Hence, whether or not sex-reversals have the capacity of the opposite of their genetically determined sex has rarely been verified. A survey of the literature on this subject revealed that only two cases of artificially induced sex-reversals producing offspring, have hitherto been reported. These sex-reversals were induced by means of castration or by implantation of gonad-preprimordium in amphibians. HARMS (1926) and PONSE (1925, 1926, 1931) showed that in castrated toads (*Bufo vulgaris*) Bidder's organs were converted into functional ovaries and that sex-reversals mated with normal males produced offspring. HUMPHREY (1945) published a brilliant study of complete and functional sex-reversal in ambystomid salamanders. He implanted the testis preprimordium of a donor embryo in the right side of a genetic female host embryo. Under the influence of a testis, the out-growth of an implanted gonadal preprimordium, the left prospective ovary-preprimordium of the host developed into a functional testis. Sex-reversals from

female to male direction thus produced were tested as breeders and produced offspring in crosses with normal females.

While there are a great number of reports of experimental inductions of sex-reversal and intersex by means of sex hormones in a series of vertebrates, presumed sex-reversals thus produced were all killed when in very young stages so were not tested as breeders. In the genetically analyzed strain of the medaka (*Oryzias latipes*) YAMAMOTO (1953) showed that complete and functional sex-reversal from male to female can be induced under the influence of estrogens. Sex-linked color genes (*R* and *r*) were used as markers of the sex-chromosomes. Genotypic females (X^rX^r) and genotypic males (X^rY^R) taken at hatching and up to the age of eight months were continuously fed estrogens. The results showed that all the genotypic males (X^rY^R) had differentiated into phenotypic females and all the genotypic females (X^rX^r) developed into females. Red females, however, may possibly be produced in ways other than sex-reversal. One way, for instance, by the effect of estrogens on the formation of orange-red pigment in white females (X^rX^r). However, we have no certain evidence of this and this way may be excluded for two reasons. (a) First, estrogenized fish were distinctly identified as white and orange-red, none being of intermediate color. (b) If estrogen-administered red fish had the genotype of X^rX^r, the F_1 offspring from mating with X^rY^R male should show the sex-ratio of 1 *r*♀ :1 *R*♂. This condition is in strong contrast to results obtained in the F_1 offspring (table 1).

Red females may also be produced by crossing over between the X^r and Y^R chromosomes in the male parent, resulting in a transfer of the gene *R* to the X from the Y. If an egg (X^r) is fertilized with an X^R spermatozoon, a red female (X^rX^R) may be produced. Although this possibility cannot be excluded, it should be noted that crossing over between the sex chromosomes X^r and Y^R is of relatively rare occurrence. Since 100 percent of the red fish treated with estrogens turned into females as previously pointed out (YAMAMOTO 1953) it is obvious that a majority, if not all, of the estrogen-treated red females described in the previous paper, might have had the genetic constitution X^rY^R. Furthermore, if these red females had the genetic constitution X^rX^R resulting from crossing over and were mated with X^rY^R males, the F_1 offspring should show the sex ratio of 1 *r*♀ :1 *R*♀ :2 *R*♂, namely the two sexes in equal numbers. This condition also is quite different from the results given in this paper (table 1).

The present progeny testing serves to endorse the fact that estrogens can induce complete and functional sex-reversal in the genotypic males (X^rY^R) of the medaka. When sex-reversed females of the male genotype (X^rY^R) are crossed with normal red males (X^rY^R), the F_1 offspring should show the sex ratio of 1 *r*♀ :3 *R*♂,—that is a preponderance of red males. The offspring would include three genotypes in the proportion of 1 X^rX^r♀ :2 X^rY^R♂ :1 Y^RY^R♂, if the zygotes of Y^RY^R genotype develop into viable males. Data presented in table 1 indicate a preponderance of red males over white females. The actual proportion of red males to white females was significantly lower than the theoretical proportion. Data suggest the rarity of YY males. This was confirmed by progeny testing of F_1 red males. Although a number of progeny tests were performed using F_1 red sons produced by mating sex-reversals with normal males for the purpose of determining either X^rY^R or Y^RY^R males, only

one fish was actually determined to be Y^RY^R male. This indicates that there were only a very few YY zygotes which survived to the time of progeny-test matings. Our Y^RY^R male mated with white females (X^rX^r) fathered all-male broods, a total of seventy-two red sons. This occurrence of a YY male producing unisexual male progeny is a valid proof that the estrone-administered red females described in our previous paper were, in fact, sex-reversed females of the male genotype (X^rY^R). Basing our judgment upon the observed 1:2.4 and 1:2.2 ratios among F_1 offspring of sex-reversals, we might expect to detect more YY males. However the fact that only a single YY male was detected may be due to the high death rate of YY males taking place between the time of our F_1-examination and the time of progeny-test matings, a period of several months.

The fact that a YY male lived and produced offspring is in striking contrast to the result obtained by BRIDGES (1916) in his study of the fruit-flx (Drosophila). He proved that YY zygotes produced by non-disjunctions were not able to survive. In contrast to many genes in the X chromosome, it is generally agreed that the Y chromosome of Drosophila contains but few genes. That YY zygotes in fish of the male heterogametic type (XY) are viable and fertile has been shown by WINGE (1934) in Lebistes and by AIDA (1936) in Oryzias. This indicates the presence of important genes in the Y chromosome of these fish other than those involved in sex determination. Later WINGE and DITLEVSEN (1938, 1947) found that in Lebistes, although $Y^{Ma}Y^{Pa}$ males (*Ma* = maculatus, *Pa* = pauper) were viable and fertile, $Y^{Ma}Y^{Ma}$ zygotes were lethal. This fact is attributable to the presence of a recessive lethal gene in the Y chromosome, according to them. Further studies are now in progress to clarify the cause of the rarity of YY males in our experiments and determined whether this is due to the semi-lethality of the YY zygotes or whether it may be attributable to the foreshortened life-span of the YY males brought about by their higher metabolic rate.

It is important to note that the normal sex-determining mechanism, XX for female and XY for male, is quickly reestablished in the F_1 offspring when sex-reversed XY females are crossed with normal XY males. The XX-XY mechanism was also generally retained by the F_2 and F_3 offspring of induced sex-reversals.

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SUMMARY

A study was made of the progeny of functional sex-reversals of the male genotype (XY) in the medaka (*Oryzias latipes*) which were induced artificially through the administration of either estrone or stilbestrol. The observed ratio of white daughters

(r♀) and orange-red sons (R♂) among F_1 offspring produced by estrone-sex-reversed mothers (X^rY^R) mated to normal orange-red males (X^rY^R) was 1:2.4 and the ratio of white daughters (r♀) and orange-red sons (R♂) among F_1 offspring produced by stilbestrol-sex-reversed mothers (X^rY^R) mated to normal orange-red males (X^rY^R) was 1:2.2, the theoretical ratio of both being 1:3.

A number of orange-red sons were singly submitted to progeny tests by crossing with white females (X^rX^r) for the purpose of determining either X^rY^R or Y^RY^R males which would be included among F_1 offspring if Y^RY^R zygotes were viable. A single orange-red son among F_1 offspring of estrone-sex-reversed mothers was identified as Y^RY^R male because in cross with white females (X^rX^r) he fathered all-male broods, a total of 72 orange-red sons. In all other progeny tests the genotype of orange-red sons was proved to be X^rY^R since they produced white females and orange-red males in the ratio of approximately 1:1.

Exceptional white males and exceptional orange-red females among F_1 and F_2 offspring were submitted to progeny testings. Many of those successfully tested were found to be the result of crossing over between the X^r and Y^R chromosomes. Two exceptional orange-red females among F_2 offspring of induced sex-reversals were detected as spontaneous sex-reversals of the male genotype (X^rY^R).

Normal sex-determining mechanism, XX for female and XY for male, is quickly reestablished when induced sex-reversed XY females are crossed with normal XY males. The XX-XY mechanism was also retained in the F_2 and F_3 offspring of artificially induced sex-reversals.

ADDENDUM

After the present manuscript was submitted, there came to my hands L. GALLIEN's brief paper entitled "Demonstration de l'homogametie du sexe mâle chez le triton *Pleurodeles waltlii* Michah. par l'étude de la descendance d'animaux à sexe physiologique inversé, après un traitement hormonal gynogène (benzoate d'oestradiol)." published in Compt. Rend. Acad. Sci. 238: 402–404, 1954. He describes having reared larvae of an urodelian amphibian (*Pleurodeles waltlii*) in solution of estradiol benzoate and obtained fully grown animals. All estrogenized animals were found to be female. Among these he was able to detect genetic males, acting as females, which in mating with normal males yielded all male offspring. This shows that the sex genotypes of the urodele are WZ for female and ZZ for male. His result indicates that functional sex-reversal from male to female direction can be induced also in Amphibia by the administration of an estrogen without a surgical operation.

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